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Anuran Amphibians: A Huge and Threatened Factory of a Variety of Active Peptides with Potential Nanobiotechnological Applications in the Face of Amphibian Decline

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1. Introduction

All anurans produce venomous skin secretions composed by a complex mixture of bioactive peptides used against potential predators and pathogens that have evolved in a predator-prey interaction and defence against a microbial invasion scenario. Each new species studied reveal new molecules, homologous to hormones, neurotransmitters, antimicrobials, as well as several others with unknown biological activity. The vast majority of species have yet to be studied. Recently, these secretions have also been reported as a rich source of multiple antimicrobial peptides against multidrug-resistant strains of bacteria, fungi, protozoa, and virus, including cancer, providing several instructive lessons for the development of new and more efficient nanotechnology based therapies for infectious disease treatment. However, new drugs arising from the identification and analysis of bioactive peptides from anuran biodiversity are threatened by amphibian decline. Nearly one-third of amphibian species are globally threatened with extinction or extinct due the effects of climate change, reduction and modification of natural habitats, pollution, as well as emerging diseases. Unfortunately, conservation efforts have not been sufficient enough to counter balance the decline in amphibian species. As a result, several species have already become extinct before their peptidome can be evaluated, and others could disappear, which would seriously inhibit understanding required for the development of important new therapies against the superbugs and degenerative diseases. This situation requires drastic strategies in order to build robust anuran peptide libraries and biological anuran tissue banks in order to conserve part of this biological richness. In this chapter, the knowledge of anuran peptide and its potential for the development of new and more effective therapies based on a nanotechnological approach against superbugs that is threatened by amphibian decline are presented.

2. Anuran amphibians: Origen, evolution and distribution

Modern amphibians belong to the subclass Lissamphibia, super order Salientia, and can be scientifically subdivided into three orders: *Anura*, which includes frogs and toads, is the largest group with more than 6,000 species; *Caudata*, which includes salamanders and newts, with 608 species; and *Gymnophiona*, the least-known group, which are commonly referred to as caecilians, with 189 species. According to the AmphibiaWeb database, numbers of new species have grown rapidly over the last 20 years or so. Since 1985 the total number of recognized species has increased by over 60%, one reflex of the growing interest in biodiversity knowledge. Currently, each new area researched shows new species, one example is the Amazon Forest, where between 1999 to 2009, 216 new species were discovered (Thompson, 2010).

The origin of amphibians can be traced back to the Devonian period (about 416 to 359 million years ago). They were developed from a common ancestor similar to the modern day coelacanth, considered as the "missing link" between fish and tetrapods (Long & Gordon, 2004). When amphibians first appeared, Earth's terrestrial area was essentially one giant landmass inhabited by plants and insects. Amphibians were the first vertebrates to make the transition from water to land (Mattoon, 2001). Somehow, a type of bony fish evolved into a creature that had four legs, could breathe atmospheric oxygen instead of dissolved oxygen, and had a body structure that allowed it to manoeuvre without the support of water (Mattoon, 2001). During the Carboniferous Period (around 359 to 299 million years ago) amphibians moved up in the food chain and occupied the ecological position that presently belongs to crocodiles. These amphibians were notable for their ability to use the mega insects on land and many types of fish as an energy source. However, during the Triassic Period (250 to 200 million years ago), the better land-adapted proto-crocodiles began to compete with amphibians for food and space (Mattoon, 2001), which, in turn, reduced their energy sources significantly, leading the amphibians to a dramatic reduction in their average size, and consequently a dropping position in the food chain. Modern anurans originated from these amphibians that had to adapt to new environment challenges in order to survive extinction.

The anuran order is the most diverse group of vertebrates, with more than 6,000 known species, a total, which is being added to annually by the discovery of new species. This order is subdivided into three suborders: *Archaeobatrachia,* which includes four families of primitive frogs*; Mesobatrachia*, which includes five families of more evolutionary intermediate frogs; and *Neobatrachia*, so far the largest group, which contains the remaining families of modern frogs, including most common species throughout the world (Table 1). The families Leptodactylidae, Hylidae and Ranidae belonging the Neobatrachia suborder are the richest in number of species.

Anurans are to be found in both tropical and subarctic regions with the exception of some ocean islands, a few deserts and Arctic and Antarctic regions (Figure 1) (Frost et al., 2008). The majority of anuran species are found in the tropical rainforests. According to the Brazilian Herpetological Society, Brazil has at least 847 anuran species (Brazilian Herpetological Society [SBH], 2011), approximately 15% of the world anuran fauna, this represents the greatest number of amphibians for any country on Earth, and is closely followed by Colombia. Both South American countries have received extensive survey efforts in recent decades, and although both countries can be expected to add significantly to their totals, the level of increase is likely to be less than in some of the other highly diverse

countries (International Union for Conservation of Nature and Natural Resources [IUCN], 2011). Within South America, Peru in particular is relatively poorly researched and is almost certain to rise very substantially in its species total (IUCN, 2011).

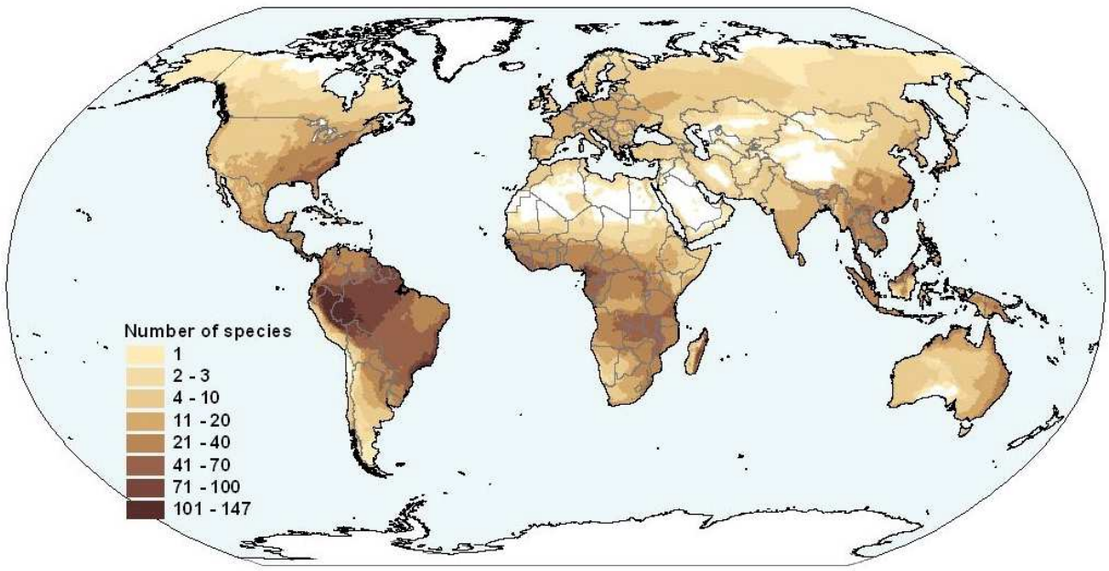

Fig. 1. Global patterns of amphibian diversity are shown. This diversity map clearly shows certain areas of high global diversity, including tropical South America and tropical West Africa. However, the problem of uneven survey efforts around the world complicates interpretation of this map. Regions such as Indonesia, New Guinea and the Congo Basin are especially likely to be under represented on this map due to lack of adequate surveys (Extracted from IUCN, 2011; Copyright 2011 International Union for Conservation of Nature and Natural Resources - Red List Unit).

3. Amphibian decline: The biodiversity crisis

According to the International Union for the Conservation of Nature (IUCN), amphibians may be the only major group currently at risk globally. IUCN assesses the status of species on a global scale and maintains a database of species that face a high risk of global extinction: the IUCN Red List of Threatened Species. The IUCN Red List, recent detailed worldwide assessment and subsequent updates show that nearly one-third of species (32.4%) are either globally extinct or threatened with extinction (Critically Endangered, Endangered and Vulnerable), representing 2,030 species (IUCN, 2011). McCallum (2007) estimates that current rates of extinction are 211 times the background extinction rate for amphibians, and rates would be as high as 25,000–45,000 times greater if all of the currently threatened species become extinct. If this is allowed to continue, the projected losses would constitute the largest mass extinction since the disappearance of the dinosaurs, which many scientists argue would be the sixth great mass extinction (Wake & Vredenburg, 2008).
Several long-term studies performed on intact natural ecosystems such as Yellowstone National Park and Sierra Nevada of California in United States (Noss et al., 2002; Vredenburg et al., 2007), Eungella National Park in Australia (McDonald, 1990), and

Monteverde Cloud Forest Preserve in Costa Rica (Pounds et al., 1997) show a worldwide decline in amphibian species in the last two decades. Populations of many species of frogs have declined dramatically in relatively undisturbed habitats at high altitudes and anthropized areas throughout the world (Blaustein & Wake, 1990, 1995; Blaustein et al., 1994; Bradford, 1991; Campbell, 1999; Carey, 1993; Collins & Storfer 2003; Crump et al., 1992; Czechura & Ingram, 1990; Hero et al., 2005; Kiesecker et al., 2001; McDonald, 1990; McMenamin et al., 2008; Pounds et al., 2006; Pounds, 2001; Reading, 2007; Richards et al., 1993; Skerratt et al., 2007; Stuart et al., 2004; Young et al., 2001). A map produced by IUCN shows the global distribution of threatened amphibians (Figure 2) revealing that the greatest concentration of threatened amphibian are in relatively limited areas dominated by species living within specific ranges, often living in mountainous areas. Many of these species have been subjected to severe habitat loss, and exposure to the fungal disease chytridiomycosis (Frost et al., 2008; IUCN, 2011).

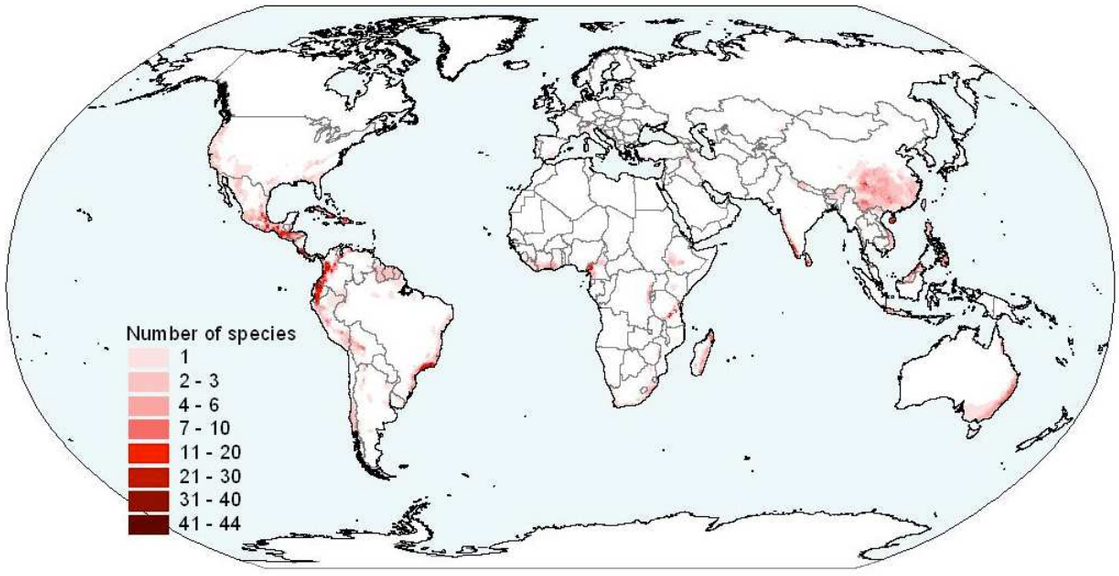

Fig. 2. Global distribution of threatened amphibians. Number of threatened species is show in red. Important concentrations of threatened species are to be found from Southern Mexico to Ecuador and Venezuela, as well as the Greater Antilles, Atlantic Forests of southern Brazil, upper Guinea forests of western Africa, forests of western Cameroon and eastern Nigeria, Albertine Rift of eastern central Africa, Eastern Arc Mountains of Tanzania, Madagascar, western Ghats of India and Sri Lanka, Borneo and Philippines, eastern Australia, central and southern China (Extracted from IUCN, 2011; Copyright 2011 International Union for Conservation of Nature and Natural Resources Red List Unit).

Atmospheric and water pollution, pathogens, exotic species, UV irradiation, and habitat destruction and/or modification have all contributed to the current amphibian decline (Alford & Richards, 1999; Blaustein et al., 2003; Collins & Storfer, 2003). Climatic change poses an additional serious threat to populations as is seen by precipitous decline of amphibian populations in remote and preserved areas. This data indicates that this phenomenon is linked to landscape and environmental changes brought about by global climatic change (Alford et al., 2007; Beebee, 1995; Carey & Alexander, 2003; McMenamin et

al., 2008; Pounds et al., 2006; Reading, 2007; Wake, 2007). According to McMenamin and co-workers (2008), changes in climate can affect amphibian populations in many ways, three of which we detail here (Figure 3).

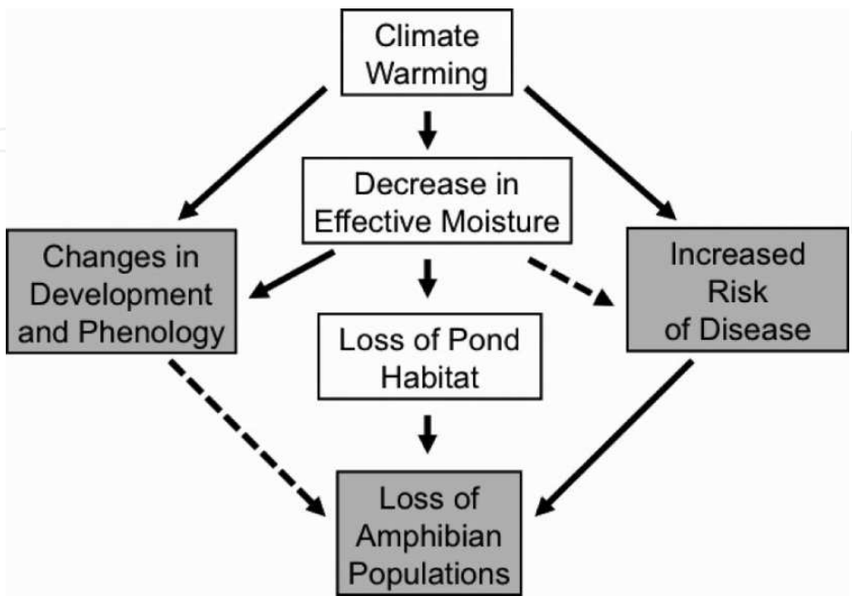

Fig. 3. Flow chart of global climatic change impacts on amphibian populations. These mechanisms are affecting amphibian populations worldwide. Solid lines denote clearly established relationships. (Extracted from McMenamin et al., 2008, Copyright 2011 National Academy of Sciences, U.S.A.).

Some examples of amphibian populations loss are catastrophic, as has been observed in Monteverde Cloud Forest Preserve in Costa Rica by Pounds and co-workers (1997) that performed a 5-year study involving daily monitoring of a large amphibian fauna demonstrating that 20 species of frogs, representing 40% of the total population, have been lost at the Preserve. What is especially notable about this case is that these observations discount the hypothesis of habitat destruction or modification, the most common reason for species disappearance, because the Preserve has a highly protected status. According to Wake & Vredenburg (2008), the start of this decline was observed in the late 1980s, where at the same time disappearances of species of the unique gastric brooding frogs from Australia (Rheobatrachus) occurred in protected areas in the Australian wet tropics (McDonald, 1990). According the same authors, at first all of these declines recorded were enigmatic, but eventually two primary causal factors emerged: the infectious disease chytridiomycosis and global warming (Lips et al., 2006; Pounds et al., 2006).

The chytridiomycosis is an emerging panzootic fungal disease caused by the chytrid fungus *Batrachochytrium dendrobatidis*. This disease was first described in 1998 from moribund and dead adult amphibians collected at sites of mass deaths in Australia and Panama between 1993 to 1998 (Berger et al., 1998). Symptoms of this amphibian lethal disease include abnormal posture, extension of hind limbs, convulsions, lethargy, and loss of attempt to escape danger; roughening of the skin; gross lesions consisting of abnormal epidermal shedding and ulceration; hemorrhages in the skin, muscle, or eye; hyperemia of digital and ventrum skin, and congestion of viscera (Berger et al., 1999; Daszak et al., 1999). According

to Berger and co-workers (1998), three mechanisms by which chytridiomycosis causes death have been proposed: epidermal hyperplasia impairs essential cutaneous respiration or osmoregulation; a fungal toxin is absorbed systemically; and a combination of both factors (Berger et al., 1998, Pessier et al., 1999).

In 2006, Pounds and co-workers hypothesized that climate change, precipitation, and increased temperature have acted synergistically in favour of the growth of the infectious chytrid fungus. This hypothesis is based on a situation where global warming has shifted temperatures closer to the presumed optimal conditions for *B. dendrobatidis*.

According the scientists of the *Intergovernmental Panel on Climate Change* (IPCC), human activities are the main cause of climate change, and will be responsible for the estimated temperature rise during the next century, that is projected to be between 2°C to 4°C, but rising as high to 7°C for much of the United States and Europe, with even higher temperatures expected in northern Eurasia, Canada, and Alaska (Parry et al., 2007). This change will produce a devastating effect on amphibian species. Impacts of the different warming scenarios are all dramatic and severe, where the first event predicted by the IPCC panel, ''Amphibian Extinctions Increasing on Mountains'', is now an empirical fact (see: http://www.ipcc.ch/graphics/ar4-wg2/jpg/ts6.jpg).

Multiple factors acting synergistically are contributing to the loss of amphibians. The association of extrinsic forces, such as global warming and increased climatic variability that increases the susceptibility of high-risk species (those with small geographic ranges, low fecundity, and specialized habitats), with habitat modification and destruction, use of fertilizers and pesticides, introduction of pollutants and exotic organisms, have severely impacted upon amphibians (Hayes et al., 2002; Sodhi et al., 2008; Wake & Vredenburg, 2008). According to Cunningham and co-workers (2006), the emergence of new infectious diseases produced by the expansion of human populations into new habitats have consequences for many other species, such as the case of chytridiomycosis in amphibians.

The IUCN has been producing lists of threatened species since the 1960s (Burton, 2003; Scott et al., 1987) reporting the very serious situation facing amphibians globally, which may be indicative of the state of freshwater species as a whole. Amphibians are declining more quickly than either birds or mammals (Stuart et al., 2004). The IUCN Red List of Threatened Species shows that at least 1,622 of the known anuran species on Earth are known to be threatened with extinction (IUCN, 2011). In 2008, a total of 120 amphibian species are listed as Critically Endangered (Possibly Extinct), and the majority of these could have disappeared since 1980 (Baillie et al., 2004; Vié et al., 2009). Because the amphibian extinctions are happening so fast and only a few areas on earth have been monitored by an insufficient number of scientists, it is difficult to obtain a complete current picture of the amphibian population status (Maas, 2011). The indications show that the extinction of amphibians is the most serious wave of all extinctions currently taking place, but the situation may be even graver than the numbers suggest (Baillie et al., 2004; Crawford et al., 2010; IUCN, 2011).

Several families of amphibians appear to be disproportionately threatened, in particular the Hynobiidae (Asian salamanders), Plethodontidae (lungless salamanders), Astylosternidae (Cameroonian stream frogs), Bufonidae (true toads), Rhacophoridae (Asian tree frogs), Leptodactylidae (typical Neotropical frogs), Leiopelmatidae (New Zealand frogs), Nasikabatrachidae (Indian burrowing frog), Rhinodermatidae (Darwin's frogs), and Sooglossidae (Seychelles frogs). Both members of the Rheobatrachidae (gastric-brooding frogs) are now Extinct, representing the loss of an entire vertebrate family (Baillie et al., 2004). It is important to note that some biologists class them within Myobatrachidae under

the subfamily Rheobatrachinae, but others place them within their own family, Rheobatrachidae (Heyer & Liem, 1976).

In spite of the massive deaths, some amphibian species appear to have an innate capacity to withstand chytridiomycosis infection. Even within species that generally succumb, some populations survive, possibly demonstrating that these anuran populations are being subjected to a selection process. According to Wake & Vredenburg (2008), despite these alarming estimates, some anuran species, particularly these that are invasive, are apparently doing very well in many parts of the world, and many thrive in landscapes heavily modified by human activities, such as the Cane Toad (*Rhinella marina*), the American Bullfrog (*Rana catesbeiana*), and the Clawed Frog (*Xenopus laevis*). They have shown they are not afflicted by chytridiomycosis.

This massive loss of anuran species diversity will produce a severe impact upon the ecosystem and human life brought about because amphibians consume huge quantities of invertebrates, including humanity's most vilified pests; play a crucial role in global ecosystems, both as predator and prey, help maintain healthy functioning environments; some species are an important protein source in many subsistence cultures and are traded in their millions as food and pets; the skin secretions that protect amphibians against predators and infection have been found to contain important pharmaceutical compounds that show potential in treating a variety of illnesses from HIV to cancer. One of these dramatic examples is the Golden Toad *Bufo periglenes* from Costa Rica, extinct since 1989 (Baillie et al., 2004), before its interesting chemical composition and potential applications could be evaluated by researches from different scientific areas.

4. Anuran skin protective adaptations

The anuran skin presents morphofunctional and behavioral protective adaptations against a number of adverse factors in the terrestrial environment (Barra & Simmaco, 2005). The cutaneous glands present in the skin play an essential role in respiration, reproduction, protection against desiccation and defence against predators and infection by microorganisms on the body surface (Toledo & Jared, 1995). Secretions produced by these glands have a key role in the protection by the presence of complex chemical composition with noxious or toxic substances with diverse pharmacological effects, which constitute an important source of biologic active compounds against bacteria, fungi, protozoa, virus and cancer (Calderon et al., 2009, 2010, 2011). However, the majority of the anuran species have not had their gland content examined by science and so remain unknown.

The cutaneous gland ultrastructural characterization of all living amphibians demonstrates that they usually belong to four main types located in the spongious dermis differing from others in size and secretory activity, and can be classified as: mucous, serous (granular or poison), lipid (or wax), and mixed (seromucous) glands (Almeida et al., 2007; Brizzi et al., 2002; Duellmann & Trueb, 1994; Lacombe et al., 2000).

Each gland presents specific action in homeostasis behavior: lipid glands promote the impermeabilization of the skin in order to decrease water loss (Castanho & De Luca, 2001); mucous glands produce mucus to support cutaneous functions, such as respiration, reproduction, thermoregulation, and defence (Toledo & Jared, 1995); serous glands, that are the largest and most widely distributed over the animal's body surface, act as a main element in amphibian passive chemical defence (Lacombe et al., 2000; Toledo & Jared, 1995). Thus, the mixed gland contains both mucous and serous cells (de Brito-Gitirana, 2004).

The serous glands produce a wide variety of noxious or toxic substances with diverse pharmacological effects on microorganisms, vertebrate, and invertebrate species (Toledo & Jared 1995; Lacombe et al., 2000). The serous glands exhibit remarkable polymorphism, having been classified into two classes, type I and II (Delfino et al., 1998; Lacombe et al., 2000). Type I glands exhibit a poorly developed smooth endoplasmic reticulum (Lacombe et al., 2000) and present two subtypes, Ia and Ib. Type Ia has dense granules that characterize the biosynthesis of proteinaceous products for exocytosis, which engage both rough endoplasmic reticulum and Golgi apparatus (Delfino, 1991). Type Ib has vesicles holding a lucent material in the fluid serous secretion on the anuran skin (Toledo & Jared, 1995). Type II glands present a well-developed smooth endoplasmic reticulum that is potentially engaged in the biosynthesis of peptides (Blaylock et al., 1976; Lacombe et al., 2000). These peptides are produced as prepropeptides, which have to be processed into mature peptides by the removal of the signal and acidic components, and then stored in the granules (Nicolas & El Amri, 2009).

Some anurans, such as the bufonidaes (toads) have a pair of peculiar glandular structures symmetrically disposed in a post-orbital position named as parotoid glands (Young, 1985). These glands are composed of large aggregations of granular glands responsible for the production and storage of a thick and creamy secretion, which contribute to protection against predators and parasites (Clarke, 1997; Croce et al., 1973; Duellman & Trueb, 1994; Sakate et al., 2000). The parotoid gland is an integument region, in which three exocrine glandular types occur: mixed glands, smaller granular glands and larger granular glands. The mixed gland is formed by mucous and serous cells while the small granular glands contain a homogene acidophilic intake. The larger granular glands produce a basophilic and alcianophilic material, and are responsible for the macroscopic protuberances designed as parotoid glands. Thus, the end product released by the parotoid glands is a mix of secretions produced by the three glands (Almeida et al., 2007).

It is accepted that the release of the gland content onto the skin surface is mediated by a holocrine mechanism that involves rupture of the plasmatic membrane and extrusion of the granules through a duct opening onto the surface (Nicolas & El Amri, 2009). Immunofluorescence analysis of *Phyllomedusa bicolor* (Hylideae) dermal glands using an antibody to the acidic propiece region of the preprodermaseptin/preprodeltorphins-derived peptide family [ENENEENHEEGSE] demonstrated that the fluorescence-positive reaction is restricted to the serous glandular content, indicating their specific role in the biosynthesis and secretion of dermaseptins and deltorphin peptides (Lacombe et al., 2000). Additionally, mass spectrometry image (MALDI-image) performed with the skin of *P. hypochondrialis* (Hylideae) indicated that the serous glands present specialization in the peptide production and storage (Brand et al., 2006b).

In spite of the large number of anuran species from different genera, a great deal of attention is being paid to the study of neotropical hylid frogs that belong to the subfamily Phyllomedusinae, as an excellent source of peptides. In 1985, Vittorio Erspamer also stated that ''No other amphibian skin can compete with that of the Phyllomedusae'' (Erspamer et al., 1985). The initial efforts on Phyllomedusa skin secretions by V. Erspamer followed by other scientists around the world during the last four decades has revealed a complex profile of biologically active peptides with antimicrobial, hormonal, and neuro activities (Calderon et al., 2011). These peptides differ significantly among species within this genus leading to an interesting molecular diversity, possibly associated with specific differences presented in the specie niche, such as interactions with the environment, predators, and

pathogens that characterize hylid species evolution (Amiche et al., 1993; Bevins & Zasloff, 1990).

5. Frog skin active peptides family: Defence against pathogens and predators

The complex chemical composition of anuran skin secretions constitutes a rich chemical warehouse of a wide number of natural biologically active compounds, such as amines, steroid derivatives, alkaloids and peptides. Peptides from anuran skin secretion are grouped into the Frog Skin Active Peptide (FSAP) family. The FSAP family can classify into three main groups according to their primary activity: antimicrobial peptides (AMPs); smooth muscle active peptides; and nervous system active peptides (Calderon et al., 2011; Erspamer et al., 1981). The secondary activities of FSAPs were not considered in this systematization. The first group acts as a skin anti-infective passive defence barrier, the second and the third groups cause the disruption of the predator homeostasis balance (Calderon et al., 2011). However, the biological activity of several peptides from anuran skin remains unknown.

The antimicrobial peptides (AMPs) compose the innate immunity system of anurans against microbial invasion (Giuliani et al., 2008; Radek & Gallo, 2007; Zasloff, 2002) effective against multidrug resistant strains of bacteria, fungi, protozoa, and virus including cancer, and provide instructive lessons for the development of new and more efficient nanotechnological-based therapies for infectious and degenerative diseases treatment (Calderon et al., 2011; Rinaldi, 2002). Many AMPs possess a wide range of activity showing effectiveness against diverse microorganism strains. One example is the dermaseptin family of AMPs and their analogs from the skin of Phyllomedusinae species. Dermaseptins have in vitro lytic activity against a broad spectrum of free-living microorganism strains, including wall-less, Gram-negative and Gram-positive bacteria, fungi, protozoa, and virus, as shown above (Table 1). Despite the sequence similarities, the dermaseptins differ in their action efficiency (Nicolas & El Amri, 2009; Rivas et al., 2009). However they present rapid and irreversible antimicrobial effect and no toxic effects in mammalian cells in vitro (Kustanovich et al., 2002; Navon-Venezia et al., 2002).

In additiony to antimicrobial activity, some dermaseptins present other additional biological functions that have unclear relations with pathogen clearance, e.g., dermaseptin B2 (adenoregulin) stimulates the binding of agonists to A1 adenosine receptors and also enhances the binding of agonists to several G-protein coupled receptors in rat brain plasmatic membrane through a mechanism involving enhancement of guanyl nucleotide exchange at G-proteins (Shin et al., 1994); Dermaseptin-B4 stimulates insulin release by acute incubation with glucose-responsive cells (Marenah et al., 2004); Dermaseptin-S1 stimulates the production of reactive oxygen species and release of myeloperoxidase by polymorphonuclear leukocytes (Ammar et al., 1998).

Gram-negative *Salmonella typhimurium,* wall-less *Mycoplasma gallisepticum* and *M. mycoides* show resistance to dermaseptin B9 from *P. bicolor* (Fleury et al., 1998).

Antimicrobial peptides are part of the innate immunity system of anurans against microbial invasion (Giuliani et al., 2008; Zasloff, 2002; Radek & Gallo, 2007). Crafted by evolution into an extremely diversified array of sequences and folds, AMPs share a common amphiphilic 3-D arrangement (Giuliani et al., 2008). This feature is directly linked to a common mechanism of action that predominantly develops upon interaction of peptides with cell membranes of target cells (Giuliani et al., 2008). The mechanisms of action of AMPs in microbial membranes are complex and still relatively unknown, but they constitute a

Microorganisms susceptible to dermaseptins	Dermaseptins active against microorganism	Species where the dermaseptin was identified	References
Wall less bacteria			
Acholeplasma laidlawii	B9	*P. bicolor*	Fleury et al., 1998
Spiroplasma apis	B9	*P. bicolor*	Fleury et al., 1998
Spiroplasma citri	B9	*P. bicolor*	Fleury et al., 1998
Spiroplasma floricola	B9	*P. bicolor*	Fleury et al., 1998
Spiroplasma melliferum	B9	*P. bicolor*	Fleury et al., 1998
Gram-negative bacteria			
Aerornonas caviae	B1	*P. bicolor*	Strahilevitz et al., 1994
	S1, S2	*P. sauvagii*	Mor & Nicolas, 1994
Acholeplasma laidlawii	B9	*P. bicolor*	Fleury et al., 1998
Acetobacter calcoaceticus	O1	*P. oreades*	Brand et al., 2002
Escherichia coli	B1, B9	*P. bicolor*	Fleury et al., 1998; Strahilevitz et al., 1994
	D1, D2, D3, D4, D5	*P. distincta*	Batista et al., 1999
	H1	*P. hypochondrialis*	Brand et al., 2006b; Conceição et al., 2006
	O1	*P. oreades*	Brand et al., 2002; Leite et al., 2008
	T7	*P. tarsius*	Silva et al., 2000
	S1, S2	*P. sauvagii*	Mor & Nicolas, 1994
Neisseria gonorrhoeae	S4	*P. sauvagii*	Rydlo et al., 2006; Zairi et al., 2009
Pseudomonas aeruginosa	B9	*P. bicolor*	Fleury et al., 1998
	D1, D2, D3, D4, D5	*P. distincta*	Batista et al., 1999
	H1	*P. hypochondrialis*	Brand et al., 2006b; Conceição et al., 2006
	O1	*P. oreades*	Brand et al., 2002; Leite et al., 2008
	T7	*P. tarsius*	Silva et al., 2000
Gram-positive bacteria			
Corynebacterium glutamicum	B9	*P. bicolor*	Fleury et al., 1998
Enterococcus faecalis	D1, D2, D3, D4, D5	*P. distincta*	Batista et al., 1999
	T7	*P. tarsius*	Silva et al., 2000
Micrococcus luteus	H1	*P. hypochondrialis*	Conceição et al., 2006
Nocardia spp	O1	*P. oreades*	Leite et al., 2008
Nocardia brasiliensis	B1	*P. bicolor*	Strahilevitz et al., 1994
	S1, S2	*P. sauvagii*	Mor & Nicolas, 1994
Staphylococcus aureus	B1, B9	*P. bicolor*	Fleury et al., 1998; Strahilevitz et al., 1994
	D1, D2, D3, D4, D5	*P. distincta*	Batista et al., 1999
	H1	*P. hypochondrialis*	Brand et al., 2006b; Conceição et al., 2006
	O1	*P. oreades*	Brand et al., 2002; Leite et al., 2008
	T7	*P. tarsius*	Silva et al., 2000
Streptococcus dysgalactiae	O1	*P. oreades*	Leite et al., 2008
Streptococcus uberis	O1	*P. oreades*	Leite et al., 2008

Microorganisms susceptible to dermaseptins	Dermaseptins active against microorganism	Species where the dermaseptin was identified	References
Fungi			
Aspergillus fumigatus	S1, S2	*P. sauvagii*	Mor & Nicolas, 1994
Arthroderma simii	B1	*P. bicolor*	Strahilevitz et al., 1994
	S1, S2	*P. sauvagii*	Mor & Nicolas, 1994
Cryptococcus neofonnans	B 1	*P. bicolor*	Strahilevitz et al., 1994
	S1, S2	*P. sauvagii*	Mor & Nicolas, 1994
Candida albicans	B 1	*P. bicolor*	Strahilevitz et al., 1994
	O1	*P. oreades*	Leite et al., 2008
	S1, S2, S4	*P. sauvagii*	Mor & Nicolas 1994; Zairi et al., 2008
Candida tropicalis	D1, D2	*P. distincta*	Leite et al., 2008
	O1	*P. oreades*	Leite et al., 2008
Candida guilliermondii	D1, D2	*P. distincta*	Leite et al., 2008
	O1	*P. oreades*	Leite et al., 2008
Microsporum canis	B1	*P. bicolor*	Strahilevitz et al., 1994
	S1, S2	*P. sauvagii*	Mor & Nicolas, 1994
Tricophyton rubrum	S1, S2	*P. sauvagii*	Mor & Nicolas, 1994
	B 1	*P. bicolor*	Strahilevitz et al., 1994
Protozoa			
Leishmania major (Pro)	S1, S4	*P. sauvagii*	Feder et al., 2000; Gaidukov et al., 2003; Kustanovich et al., 2002
Leishmania mexicana (Pro)	S1	*P. sauvagii*	Hernandez et al., 1992; Mor & Nicolas 1994b
Leishmania amazonensis (Pro)	O1	*P. oreades*	Brand et al., 2006b
	H1	*P. hypochondrialis*	Brand et al., 2006b
Leishmania amazonensis (Epi)	H5	*P. hypochondrialis*	Brand et al., 2006b
Leishmania chagasi (Pro)	H5	*P. hypochondrialis*	Zampa et al., 2009
Plasmodium falciparum (Trf)	S3, S4	*P. sauvagii*	Ghosh et al., 1997; Krugliak et al., 2000
Trypanosoma cruzi (Try)	O1	*P. oreades*	Brand et al., 2002
	D1, D2	*P. distincta*	Brand et al., 2002
Virus			
HSV-1	S4	*P. sauvagii*	Belaid et al., 2002
HIV-1	S4	*P. sauvagii*	Lorin et al., 2005; Zairi et al., 2009

Table 1. Microorganisms susceptible to dermaseptins from anuran species belonging the genus Phyllomedusa.

promising and attractive proposition as new antimicrobial therapeutics (Calderon et al., 2009, 2010, 2011). Interestingly, the mechanism of interaction between AMP and microbial membrane inhibits a fast adaptation of parasites to the peptide action, as it requires a wide change in its membrane structure or composition, demanding a significant great metabolic change in a short period of time, in contrast to drugs of intracellular action (Phillips, 2001).
The emergence, increased prevalence and rapid spread of extremely multidrug resistant pathogenic microorganisms together with the increased use of immunosuppressive

therapies, and the association with HIV co-infection present a serious challenge to public health systems around the world. The lack of therapeutic options against these pathogens has stimulated research into new bioactive molecules from the biodiversity as a source of more efficient (low toxicity and major potency) mechanisms for infection control (Calderon et al., 2009; Vaara, 2009).

The interest in the development of new forms of anti-infective agents such as those based on AMPs from anuran skin as therapeutic agents has been increased (Rinaldi, 2002; Xiao et al., 2011). Thus, they are likely to be active against pathogens and even those that are resistant to conventional drugs. Many peptides have been isolated and shown to be effective against multi-drug resistant pathogens. According to Xiao and co-workers (2011), more than 500 AMPs have been identified from amphibians. This number of peptides described is insignificant when compared to all the potential represented by the amphibian global fauna, that are composed of much more than 6,000 species, with increases new species every year. According to Jared & Antoniazzi (2009), from the toxinology viewpoint, is possible imagine that with more than 6,000 species, should be at least 6,000 kinds of poison and hundreds of thousands of new bioactive molecules to be discovered.

Of a total of 49 anuran families (Frost, 2011) only 10 have had part of their peptides identified, as can be observed in Table 2. Only members of the Ascaphidae, Bombinatoridae, Hylidae, Hyperoliidae, Leiuperidae, Leptodactylidae, Myobatrachidae, Pipidade, Ranidae families have been examined in order to discover new bioactive peptides. Members of the families Hylidae and Ranidae have received more attention, with a high number of peptide families characterized. The abundance of AMPs in frog skin is remarkable and constitutes a rich source for the design of new pharmaceutical molecules. Unfortunately, several anuran species have become extinct due to the events related to the amphibian decline before their bioactive molecules have had a chance to be discovered, such as the golden toad *Bufo periglenes* (Bufonidae) (Figure 4).

Suborder	**Family (conservation status)**	**Peptide identified**
Archaeobatrachia	Ascaphidae	Ascaphin, Bradykinin, Skin secreted peptide, Tryptophyllin
	Bombinatoridae	Bombesin, Bombinin, Bradykinin, Maximin, Tryptophyllin, Thyroliberin
	Discoglossidae (**EX**)	Alytesin
	Leiopelmatidae (**CR**)	none
Mesobatrachia	Megophryidae (**CR**)	none
	Pelobatidae	none
	Pelodytidae	none
	Pipidae (**CR**)	Antimicrobial peptide, Caerulein, Dorphin, Leap2 protein, Levitide, Magainin, Midkine, Midkine, Peptide pGQ, Peptide PYLa/PGLa, Pleiotrophin, Xenopsin, Xenoxin
	Rhinophrynidae	none
	Scaphiopodidae	none

Suborder	Family (conservation status)	Peptide identified
Neobatrachia	Allophrynidae	none
	Aromobatidae (**CR**)	none
	Arthroleptidae (**CR**)	none
	Brachycephalidae	none
	Brevicipitidae	none
	Bufonidae (**EX**, **EW**, **CR**)	Neurotensin, Seritocin
	Calyptocephalellidae (**CR**)	none
	Centrolenidae (**CR**)	none
	Ceratobatrachidae (**CR**)	none
	Ceratophryidae (**CR**)	none
	Ceuthomantidae	none
	Craugastoridae (**EX**, **CR**)	none
	Cycloramphidae (**CR**)	none
	Dendrobatidae (**CR**)	none
	Dicroglossidae (**EX**, **CR**)	none
	Eleutherodactylidae (**CR**)	none
	Heleophrynidae (**CR**)	Bradykinin
	Hemiphractidae (**CR**)	none
	Hemisotidae	none
	Hylidae (**EX**, **CR**)	Antimicrobial peptide, Aurein, Bioactive peptide, Bradykinin-potentiating peptide, Bradykinin, Caeridin, Caerin, Caerulein, Citropin, Dahlein, Deltorphin, Dermadistinctin, Dermaseptin, Dermatoxin, Dermorphin, Electrin, Fallaxidin, Frenatin, Hylaseptin, Hylin, Hyposin, Litorin, Maculatin, Novel peptide, Peptide TRP, Peroniin, Phyllocaerulein, Phyllokinin, Phyllomedusin, Phylloseptin, Pseudin, Rothein, Rubellidin, Skin secreted peptide, Splendipherin, Tryptophyllin, Uperin
	Hylodidae (**CR**)	none
	Hyperoliidae (**CR**)	Caerulein-like, FMRFamide-related, Galensin, Hylambatin, Kasseptin, Kassinakinin, Kassinatuerin, Kassinin, Kassinin, Kassorin, Tachykinin
	Leiuperidae (**CR**)	Bradykinin, Phyllokinin, Physalaemin
	Leptodactylidae (**CR**)	Aggression-stimulating peptide, Leptoglycin, Ocellatin, Ranaspumin
	Limnodynastidae (**CR**)	none
	Mantellidae (**CR**)	none

Suborder	Family (conservation status)	Peptide identified
Neobatrachia	Micrixalidae (**CR**)	none
	Microhylidae (**CR**)	none
	Myobatrachidae (**EX**, **CR**)	Bombesin, Crinia-angiotensin, Deserticolin, Dynastin, Fletcherin, Kassinin, Riparin, Rugosauperolein, Signiferin, Substance P-like, Uperin, Uperolein
	Nasikabatrachidae	none
	Nyctibatrachidae	none
	Petropedetidae (**CR**)	none
	Phrynobatrachidae	none
	Ptychadenidae	none
	Pyxicephalidae (**CR**)	none
	Ranidae (**EX**, **CR**)	Atrial natriuretic factor, Bombesin, Bradykinin, Brevinin, Calcitonin, Chensinin, Gaegurin, Galanin, Granuliberin, Guentherin, Hydrin, Japonicin, Lectin-like, Melittin-like, Neurokinin, Neuromedin, Neurotensin, Nigrocin, Odorranain, Orexigenic neuropeptide, Palustrin, Peptide tyrosine arginine, Ranacyclin, Ranakinin, Ranalexin, Ranamargarin, Ranatachykinin, Ranatensin, Ranatuerin, Rugosin, Temporin, Tigerinin, Vasoactive intestinal peptide
	Ranixalidae (**CR**)	none
	Rhacophoridae (**EX**, **CR**)	none
	Sooglossidae	none
	Strabomantidae (**CR**)	none

*According to the IUCN (2011), of the 6,260 amphibian species assessed, nearly one-third of species (32.4 %) are globally threatened or extinct, representing 2,030 species. Thirty-eight of the 2,030 species are considered to be Extinct (EX), and one Extinct in the Wild (EW). Another 2,697 species are not considered to be threatened at present, being classified in the IUCN Categories of Near Threatened or Least Concern, while sufficient information was not available to assess the status of an additional 1,533 species. It is predicted that a significant proportion of these Data Deficient species are likely to be globally threatened (IUCN, 2011; Frost et al., 2008).

Table 2. Anuran families ordered by suborder according to Frost (2011) with current status informed by IUCN* and peptide family described for each one deposited in the UniProtKB/Swiss-Prot. Current status are designated by the presence of extinct species (**EX**), extinct species in the wild (**EW**), and/or critically endangered species (**CR**) according to Frost and co-workers (2008).

Since the first peptide was isolated from the Phyllomedusa skin, the Phyllokinin, a bradykinyl-isoleucyl-tyrosine O-sulfate from *P. rohdei* in 1966 by Erspamer's research group (Anastasi et al., 1966), the number of anuran peptides discovered has increased exponentially (Calderon et al., 2011), but is still far from its real potential, which is evidenced by the observation that for every new anuran species studied new peptides are found, with homologies to hormones, neurotransmitters, antimicrobials, and several other peptides with unknown biological activity.

Fig. 4. Golden toad *Bufo periglenes* (Bufonidae) (**EX**) from Costa Rica (male), also called the Monteverde golden toad, or the Monte Verde toad. First described in 1966 (Savage, 2002), is considered extinct by the IUCN since 1989 (IUCN, 2011), before its content of bioactive molecules could be researched (Image from the U.S. Fish and Wildlife Service's online digital media library, public domain).

Nowadays, it is possible to carry out transcriptome analysis in order to build a robust cDNA library only with the secretions from a single living specimen (Chen et al., 2003b). The emergence of modern high-throughput molecular technologies involving *de novo* peptide sequencing via tandem mass spectrometry, cDNA cloning, and pharmacological screening applied to peptide discovery allowed fast structural data analysis and the generation of peptide sequence libraries, which in turn increased the capacity of peptide characterization, thus reducing the amount of samples needed (Shaw, 2009), which reduces significantly the impact on the amphibian populations researched by the reduction of the number of individuals necessary to perform bio prospection research.

6. The resistance crisis: Increasing need for new antimicrobials

Recently, antibiotic-resistant infections have reached unprecedented levels, some public health specialists and scientists have been warning that the antibiotic-resistant microorganisms strains, or superbugs, outstrip our ability to fight them with existing drugs. It is estimated that in 2007 approximately 25,000 patients died in the European Union, Iceland and Norway from an infection due to antibiotic-resistant bacteria that is able to outsmart even the newest antibiotics, such as *Staphylococcus aureus*, methicillin resistance (MRSA); *S. aureus*, vancomycin intermediate resistance and vancomycin resistance

(VISA/VRSA); *Enterococcus* spp. (e.g. *Enterococcus faecium*), vancomycin resistance (VRE); *Streptococcus pneumoniae*, penicillin resistance (PRSP); Enterobacteriaceae (e.g. *Escherichia coli*, *Klebsiella pneumoniae*), third-generation cephalosporin resistance; Enterobacteriaceae (e.g. *K. pneumoniae*), carbapenem resistance; and Non-fermentative Gram-negative bacteria (e.g. *Pseudomonas aeruginosa*), carbapenem resistance. In addition, infections due to any of these antibiotic-resistant bacteria resulted in approximately 2.5 million extra hospital days and extra in-hospital costs of more than EUR 900 million (European Centre for Disease Prevention and Control/European Medicines Agency [ECDC/EMEA] Joint Working Group, 2009). The situation has reached to a critical point.

One example of this situation is the emergence and rapid spread of extremely multiresistant pathogenic microorganisms endowed with new antibiotic resistance mechanisms such as *New Delhi metallo-beta-lactamase-1* (NDM-1), an enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics, including the carbapenem family of antibiotics (except aztreonam), one of last resort for many bacterial infections, such as *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (Kumarasamy et al., 2010; Nordmann et al., 2011; Richter et al., 2011). This gene has been identified in strains that possess other resistance mechanisms contributing to their multidrug resistance patterns, because these bacteria have often been referred to in the news media as "superbugs" because infections caused by them are difficult to treat successfully (Raghvendra et al., 2011). Most isolates with NDM-1 enzyme are resistant to all standard intravenous antibiotics for treatment of severe infections (Health Protection Agency [HPA], 2009a,b). It has been recently extensively reported from the United Kingdom, India and Pakistan and, albeit to a lesser extent, from a number of other countries worldwide (Nordmann et al., 2011).

The emergence of multiresistant pathogenic microorganisms, increased use of immunosuppressive therapies, and the association with HIV co-infection, represent a serious public health problem with high mortality and morbidity rates, such as *Cryptococcus*, *Cryptosporidium* and *Leishmania* (Abu-Raddad et al., 2006; Pukkila-Worley & Mylonakis, 2008; Rivas et al., 2009; Vaara, 2009). The critical problem represented by the limited therapeutic options for increasing multidrug resistance in Gram-negative bacteria, in particular *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*, have forced infectious disease clinicians and microbiologists to reappraise the clinical application of polymyxin antibiotic, a cyclic peptide with a long hydrophobic tail, discovered more than 50 years ago (Li et al., 2006). Polymyxin is usually active *in vitro* (though not *vs. Morganella morganii*, an intrinsically resistant species) but of uncertain clinical efficacy, especially in pneumonia, owing to poor lung penetration. The Antibiotic Resistance Monitoring & Reference Laboratory (ARMRL) from the HPA Centre for Infections with the pharmaceutical industry is urgently reviewing the activity of both experimental and outdated antibiotics in order to develop alternative chemotherapies for NDM-1 (HPA, 2009a,b).

One of the greatest accomplishments of modern medicine has been the development of antibiotic therapies for potentially fatal infections by multidrug-resistant pathogenic microorganisms. Unfortunately, over the past two decades, the discovery and development of novel antibiotics has decreased while pathogen resistance to those currently available has increased (Li et al., 2006).

According to the European Centre for Disease Prevention and Control (ECDC) and the European Medicines Agency (EMEA) with contributions from the international network Action on Antibiotic Resistance (ReAct), there is a need for more development of antibiotics that are effective against multidrug-resistant bacteria. The ECDC/EMEA Joint Working

Group, using data from the European Antimicrobial Resistance Surveillance System (EARSS) and two commercial databases of antibacterial agents in clinical development worldwide (Adis Insight R&D and Pharmaprojects) concluded that there is a gap between the burden of infections due to multidrug-resistant bacteria and the development of new antibiotics to tackle the problem; and that a European and global strategy to address this gap is urgently needed (ECDC/EMEA Joint Working Group, 2009).

Limited therapeutic options against these pathogens demand urgent prospection of new bioactive molecules from the biodiversity as a source for more efficient (low toxicity and major potency) mechanisms of microorganism killing (Calderon et al., 2009; Vaara, 2009). The discovery of new lead compounds is important to subsidize the development of new chemicals with structural characteristics for large-scale production by the pharmaceutical industry at a feasible cost. The sources from the biodiversity, such as the skin of several amphibian and other vertebrate and invertebrate animals, plants, and microorganisms, have proved to be an inexorable source of antimicrobial molecules, with a broad spectra of activity (Calderon et al., 2009), specially against the drug-resistant pathogens described before, in which the AMPs have highlights in their potential therapeutical application as exposed in Table 1 (Gomes et al., 2007; Hancock, 1997; Hancock & Lehrer 1998; Koczulla & Bals, 2003).

One interesting example is the cationic alpha-helical peptide Ascaphin-8 (GFKDLLKGAAKALVKTVLF-NH2), from the skin secretion of the primitive anuran *Ascaphus truei* (Archaeobatrachia: Ascaphidae) (Conlon et al., 2004). This AMP shows broad-spectrum antibacterial activity against clinical isolates of beta-lactamase producing bacteria such as *Escherichia coli* (MIC=1.5-6 microM) and *Klebsiella pneumoniae* (MIC=12.5-25 microM), as well as a group of miscellaneous beta-lactamase producing strains of *Citrobacter*, *Salmonella*, *Serratia*, and *Shigella* spp (Eley et al., 2007). According to Eley and co-workers (2007), Ascaphin-8 is also toxic to human erythrocytes ($LC_{(50)}$= 55 microM), however, the L-lysine-substituted analogs Lys10, Lys14, and Lys18 also displayed potent antibacterial activity while showing very low hemolytic activity ($LC_{(50)}$> 500 microM). This result shows that peptide engineering could reduce toxicity of haemolytic AMPs, which makes possible the development of a drug delivery system association to improve the efficiency of Ascaphin-8 analogs to be used as a therapeutic peptide antibiotic against multidrug-resistant pathogenic microorganisms.

7. Peptide antibiotics: Nanotechnological approaches against superbugs

According to Marr and co-workers (2006), therapeutic peptide antibiotics will have advantages over conventional antibiotics due to their diverse potential applications, such as single antimicrobials, in combination with other antibiotics for a synergistic effect, or as immunomodulatory and/or endotoxin-neutralizing compounds (Zasloff, 2002). In particular, the most potent agents have an unusually broad spectrum of activity against most Gram-negative and Gram-positive bacteria, and also to fungi and even a variety of viruses, such as dermaseptins (Table 1). One of their advantages is their ability to kill multidrug-resistant bacteria (Marr et al., 2006). Compared with conventional antibiotics, these bacteria-killing peptides are extremely rapid and attack multiple bacterial cellular targets (Brogden, 2005).

Despite their obligatory interaction with the plasmatic membrane, some peptides are able to perforate plasmatic membrane at their minimal inhibition concentration (MIC), a number of AMPs translocate across the membrane and affect cytoplasmic processes, including

inhibition of macromolecular synthesis, particular enzymes or cell division, or the stimulation of autolysis (Marr et al., 2006). Minimal inhibitory concentrations and minimal bactericidal concentrations often coincide (less than a two-fold difference), indicating that killing is generally bactericidal, a highly desirable mode of action (Marr et al., 2006). Furthermore, AMPs are not hindered by the resistance mechanisms that occur with currently used antibiotics (Zhang et al., 2005). Indeed, killing can occur synergistically with other AMPs and conventional antibiotics, helping overcome some barriers that resistant bacteria have against currently used antibiotics (Marr et al., 2006).

Until then, many efforts have been carried out in order to use the AMPs in the development of new infection-fighting drugs applicable to new treatments of nosocomial infections and multidrug-resistant infections (Amiche et al., 2000), due to the skill of the AMPs to kill multidrug resistant strains of microorganism by a mechanism unlikely to induce antibiotic-resistance. The development of new antimicrobials based on AMPs hold promises to medicine at the end of the classical antibiotic age by the emergence of the multidrug-resistant microorganisms (Alanis, 2005; Arias & Murray, 2009; Nordmann et al., 2011).

Even with the expected advantages in the use of AMPs as new antimicrobials for the post-antibiotic age, several impediments to therapeutic peptides arise. According to Marr and co-workers (2006), the main problem at the present moment is the cost of manufacturing peptides, which is economically unfeasible for the amounts of AMPs needed compared to other antibiotics, preventing the widespread clinical use of AMPs as a common antibiotic, and the shortage of studies thoroughly examining systemic peptide pharmacodynamic and pharmacokinetic issues, including peptide aggregation problems, the in vivo half-life of peptides (and particularly their susceptibility to mammalian proteases), and the required dosing frequency (Marr et al., 2006). Due to the specific characteristics of the AMPs, that differentiate them from other antibiotics, the development of new strategies for the therapeutic use of AMPs in medicine are necessary in order to reduce the amount of AMPs necessary to promote the therapeutic infection suppression effect, including the addition of striking affinity to specific targets, efficiency at very low concentrations and negligible toxicity (Marr et al., 2006).

From this viewpoint, the nanotechnological approach has become an efficient and viable alternative to promote the therapeutic application of AMPs by the use of nanocarriers in order to: protect the AMP from degradation; enhance AMP absorption by facilitating diffusion through epithelium; modification of pharmacokinetic and tissue distribution profile; and/or improving intracellular penetration and distribution.

According to Couvreur & Vauthier (2006), over the past 30 years, the explosive growth of nanotechnology has burst into challenging innovations in pharmacology, which is in the process of revolutionizing the delivery of biologically active compounds.

The main application of nanotechnology in cancer and infectious diseases pharmacology collaborate with the development of several approved forms of drug-targeting systems for the treatment of certain cancer and serious infectious diseases (Couvreur & Vauthier, 2006). One of the main examples is the Ambisome®, a formulation of amphotericin B in liposome, which was marketed in 1996 (NeXstar now Gilead, Foster City, CA, USA). Before the nanostructured formulation, the toxicity of the leading compound against leishmaniasis and fungus, was 50- to 70-fold higher (Adler-Moore & Proffitt, 1993). This allowed the administration of more than 5-fold of the drug compared with conventional treatments. Thus, today it is considered the most efficient treatment for leishmaniasis and other fungal infections (Dupont, 2002; Ringden, 2002; Croft & Coombs, 2003).

Nanotechnology also seems to be a promising alternative to overcome the problems of the administration of peptides and of the new drug molecules coming out of the discovery pipeline. Nanotechnological based drug-targeting system carrying AMPs can be targeted to a precise location which would make the AMP much more effective, reducing the amount necessary to promote the antimicrobial action, as well as the chances of possible side-effects and production costs, making the therapeutic peptide antibiotics feasible economically compared to other antibiotics.

In recent years, significant efforts have been devoted to the development of nanotechnological tools capable of enhancing the assembly and immobilization of AMPs in a synergistic way in biomedical devices (Huguenin et al., 2005; Siqueira et al., 2006; Zampa et al., 2007; Zucolotto et al., 2006, 2007).

The structural and physico-chemical properties of the AMPs, such as the presence of a a-helix fold and distribution of positive charges along the chain have allowed their use as active material in the development of bio-nanostructures with a potential application in therapeutics by the pharmaceutical industry and diagnosis (Zampa et al., 2009). These nanostructures include cationic nanoparticles, formed by the conjugation of cholesterol and AMPs, able to cross the blood–brain barrier for treatment of fatal Cryptococcal meningitis in patients with late-stage HIV infection (Wang et al., 2010); Polymyxin B conjugates with Au nanoparticles and CdTe quantum dots with improved antimicrobial activity and reduced toxicity to mammalian cells (Park et al., 2011); nanostructured thin films with immobilized AMPs as an agent intended to combat and prevent infection and formation of Staphylococcus biofilm (slimelike communities) related implant failure (Shukla et al., 2009); or as sensor elements for detection of Leishmania cells using cyclic voltammetry (Zampa et al., 2009).

The use of the AMPs through nanotechnological innovation approach could provide an entirely novel way to treat and prevent infection and new systems for the detection and identification of infectious parasites. Nanotechnology could provide new ways to use lower amounts of AMPs with extreme efficiency in the infection suppression, by improving the cell, tissue, or organ's specific biodistribution and increasing AMP potency by the association with nanotechnological structures. It is expected that in the forthcoming years nanotechnology will promote the emergence of new products for control and prevention of multidrug-resistance microbe infection arising from the identification and analysis of AMPs from anuran amphibian biodiversity.

8. Final considerations

Anuran amphibians are an enormous source of bioactive molecules with potential application for the development of new nanotechnological based therapies against multidrug-resistant microorganisms in the modern day public health system crisis. However, the emergence of chytridiomycosis, climate change, pollution, and destruction and/or alteration of natural habitats are producing a devastating effect on biodiversity causing the amphibian decline. One-third of the anuran species are extinct or threatened with extinction before its content of bioactive molecules, specially the antimicrobials, can be discovered. Without a concerted effort, biodiversity and humans could be dealing with the "nightmare scenario" of a worldwide spread of untreatable infections and disappearance of species with potential solutions to combat superbugs. A united push to inspect and preserve the biodiversity in order to produce subsides for the development of new drugs is urgently needed.

9. Abbreviations

AMP	Antimicrobial peptide
ARMRL	Antibiotic Resistance Monitoring & Reference Laboratory
CR	Critically Endangered
EARSS	European Antimicrobial Resistance Surveillance System
ECDC	European Centre for Disease Prevention and Control
EMEA	European Medicines Agency
Epi	Epimastigote form
EW	Extinct in the Wild
EX	Extinct
FSAP	Frog Skin Active Peptide
HIV-1	Human Immunodeficiency Virus 1
HPA	Health Protection Agency
HSV-1	Herpes Simplex Virus 1
IPCC	Intergovernmental Panel on Climate Change
IUCN	International Union for Conservation of Nature and Natural Resources
$LC_{(50)}$	Lethal Dose, 50%
Lys	Lysine
MALDI-image	Matrix Assisted Laser Desorption Ionization-Image
MIC	Minimal Inhibition Concentration
MRSA	*Staphylococcus aureus*, methicillin resistance
NDM-1	New Delhi metallo-beta-lactamase-1
Pro	Promastigote form
PRSP	*Streptococcus pneumoniae*, penicillin resistance
Trf	Trophozoite form
Try	Trypomastigote form
UniProt	The Universal Protein Resource
VISA	*Staphylococcus aureus*, vancomycin intermediate resistance
VRE	*Enterococcus faecium*, vancomycin resistance
VRSA	*Staphylococcus aureus*, vancomycin resistance

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As everybody knows, the dynamic interactions between biotic and abiotic factors, as well as the anthropic ones, considerably affect global climate changes and consequently biology, ecology and distribution of life forms of our planet. These important natural events affect all ecosystems, causing important changes on biodiversity. Systematic and phylogenetic studies, biogeographic distribution analysis and evaluations of diversity richness are focal topics of this book written by international experts, some even considering economical effects and future perspectives on the managing and conservation plans.

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