16: [illegible]

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Cysteine-rich secretory proteins (CRISPs) are found in a wide variety of animal tissues, particularly the epididymis of mammals, and most reptile venoms appear to contain at least one isoform. Although several venom CRISPs have been assigned speci c functions, many have not, and the biological signi cance of this family of proteins in venoms is not clear. In many colubrid venoms, they are major protein constituents, suggesting that they have an important role in envenomation. Like many other families of reptile toxins, CRISPs show a highly conserved molecular scaffold, and the sixteen cysteines and eight disul des they form are 100% conserved. Because they are widely distributed among reptile venoms, show structural conservation, and many have been sequenced, they may have utility as phylogenetic markers. In general, venom CRISP relationships re ect established phylogenetic relationships among the species from which they are derived. By analogy with the three- nger toxins of reptile venoms, which also have a highly conserved protein scaffold sta-

Schambony et al., 1998, 2003; Roberts et al., 2006). Although the function of many of the CRISPs

CRISPs currently have no identi able function and apparently no acutely toxic effects (Chang et al., 1997; Yamazaki et al., 2002b; Jin et al., 2003; Osipov et al., 2005; Heyborne and Mackessy, unpublished data).

Several reptile venoms have contained multiple CRISP isoforms (Jin et al., 2003, Osipov et al., 2005; Fry et al., 2006). With this in mind, it would be interesting to examine the venom of *Heloderma horridum* more thoroughly. The CRISP from the venom of this species (helothermine) has very diverse functionalities, including the blockage of multiple types of ion channels (Nobile et al., 1994, 1996) and the induction of hypothermia in prey (Mocha-Morales et al., 1990). Given the diversity of biological activities reported for helothermine, one might hypothesize there to be more than a single CRISP isoform in the venom of this species, each with a slightly different sequence and thus biological activity.

Despite the lack of functional data for many of the CRISPs, the structural chemistry of the venom CRISPs is quite well understood, following the recent crystallization of three such molecules (Guo et al., 2005; Shikamoto et al., 2005; Wang et al., 2005). These venom CRISP structures have shown this family of proteins to have a highly conserved primary, secondary, and even tertiary structure. Due to the high levels of structural conservatism, new members of this family are easily identi able based on their primary structure alone.

Cysteine-rich secretory proteins were rst named because of the large number of cysteine residues found in the C-terminal portion (the cysteine-rich domain—see below). However, because many venom proteins contain numerous cysteines and disul des, Kini et al. (2001) suggested the name *helveprin* (derived from *helothermine-like venom protein*) to distinguish venom CRISPs from other cysteine-rich venom proteins. Like the phospholipases A_2 (PLA_2s) and three- nger toxins (3FTxs) (see Chapters 5 and 10, this volume), venom CRISPs have a constrained structure de ned by sixteen cysteines participating in eight highly conserved disul de bonds (Table 16.1).

The rst comprehensive structural analysis of a venom CRISP was conducted on the protein stecrisp from the venom of *Trimeresurus stejnegeri* (Guo et al., 2005). Crystallization of this molecule showed stecrisp to be comprised of two distinct regions connected by a folded hinge or bridge (Figure 16.1). The rst of these regions, from the N-terminus of the molecule, was called the PR-1 domain due to its structural homology to the plant pathogenesis group 1 protein family. Known PR-1 crystal structures, including P14a described by Fernández et al. (1997), have shown a characteristic α/β/α sandwich element, which was also seen in stecrisp. The second region, from the C-terminal portion of stecrisp, was called the cysteine-rich domain (CRD) due to the high proportion of cysteine residues in this part of the molecule. Previous work on venom CRISPs had shown a strictly conserved set of sixteen cysteine residues throughout the molecule (Yamazaki and Morita, 2004). Guo et al. (2005) showed these sixteen residues form eight paired disul de bonds in stecrisp. Three of these were found in the PR-1 domain, two in the hinge or bridge, and three in the cysteine-rich domain. Subsequent crystallization of two additional venom CRISPs (natrin from the venom of *Naja atra*, Wang et al., 2005, and tri in from *Trimeresurus avoviridis*, Shikamoto et al., 2005) con rmed the presumed structural homology of venom CRISPs, as natrin and tri in also showed the two bridge-connected domains, as well as the α/β/α sandwich element in the PR-1 domain and the eight conserved disul de bonds.

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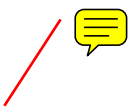
et al., 2003; Fry and Wüster, 2004) have recognized the potential use of CRISPs and have used CRISP sequence data in phylogenetic analyses. However, the number of CRISP sequences now available has grown signi cantly. A BLAST search of available sequences (2007) revealed forty-nine CRISP sequences, most of which are derived from reptile venom gland DNA sequences (see Table 16.1 and appendix). These forty-nine sequences were aligned and a neighbor-joining tree was drawn using ClustalX 1.81 and TreeView 1.6.6. (Figure 16.2). In general, sequence similarities follow phylogenetic af nities, with an exception that two elapid taxa (



GenBank accession number, trivial name, and source species are provided for each CRISP.

B0WCQ0	Catrin	*Culex quinquefasciatus* (southern house mosquito)
Q642T6	Crisp2 protein	*Xenopus tropicalis* (western clawed frog)
Q5BL94	MGC108118 protein	*Xenopus tropicalis* (western clawed frog)
Q801Z0	Cysteine-rich secretory protein	*Xenopus laevis* (African clawed frog)
Q91055	Helothermine precursor (HLTx)	*Heloderma horridum horridum* (Mexican beaded lizard)
Q2XXP2	CRISP-VAR10 (fragment)	*Varanus varius* (lace monitor)
Q2XXR2	CRISP-VAR3 (fragment)	*Varanus acanthurus* (ridge-tailed monitor)
Q2XXR1	CRISP-VAR4 (fragment)	*Varanus acanthurus* (ridge-tailed monitor)
Q2XXR0	CRISP-VAR5 (fragment)	*Varanus acanthurus* (ridge-tailed monitor)

Colubridae

Q8JGT9	Tigrin precursor	*Rhabdophis tigrinus tigrinus* (tiger keelback snake)
Q2XXP4	CRISP-TRI1 (fragment)	*Trimorphodon biscutatus* (lyre snake)
Q2XXQ6	CRISP-DIS1	*Dispholidus typus* (boomslang)
Q2XXQ5	CRISP-DIS2	*Dispholidus typus* (boomslang)
Q2XXQ4	CRISP-DIS3	*Dispholidus typus* (boomslang)
Q09GJ9	Cysteine-rich secretory protein precursor (CRISP-PHI1) (CRISP-PHI2)	*Philodryas olfersii* (green snake)
Q2XXQ3	CRISP-ENH1	*Enhydris polylepis* (Macleay's water snake)
Q2XXQ2	CRISP-ENH2	*Enhydris polylepis* (Macleay's water snake)
Q2XXP5	CRISP-TEL1 (fragment)	*Telescopus dhara* (Egyptian catsnake)
Q2XXQ1	CRISP-LEI1 (fragment)	*Leioheterodon madagascariensis* (Malagasy giant hognose snake)
Q2XXQ0	CRISP-LIO1 (fragment)	*Liophis poecilogyrus* (water snake)

Viperidae

A7X4T8	CRISP-Cau1 (fragment)	*Causus rhombeatus* (rhombic night adder)
Q8JI40	Ablomin precursor	*Agkistrodon halys blomhof* (mamushi) (*Gloydius blomhof i*)
Q8JI39	Tri in precursor	*Trimeresurus avoviridis* (Habu) (*Protobothrops avoviridis*)
P60623	Cysteine-rich secretory protein precursor (Stecrisp)	*Trimeresurus stejnegeri* (Chinese green tree viper)
P79845	Cysteine-rich venom protein precursor (TM-CRVP)	*Trimeresurus* (*Protobothrops*) *mucrosquamatus* (Taiwan habu)
Q7ZZN9	Cysteine-rich venom protein precursor (TJ-CRVP)	*Trimeresurus* (*Protobothrops*) *jerdonii* (Jerdon's pit-viper)
Q7ZT99	Catrin-1/2 precursor	*Crotalus atrox* (western diamondback rattlesnake)
B0VXV6	Cysteine-rich secretory protein isoform 2	*Sistrurus catenatus edwardsii* (desert massasauga)
Q7ZTA0	Piscivorin precursor	*Agkistrodon piscivorus piscivorus* (eastern cottonmouth)

Elapidae

Q7ZT98 Ophanin precursor (Opharin) *Ophiophagus hannah* (king cobra)
Q7T1K6