Handle with care! Morphology of spines and milking practices in venomous fishes

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Abstract

Venomous fish have independently evolved venom-delivery systems multiple times throughout their evolution. Despite the remarkable convergence of such structures, a large variety in venom-delivery structures morphology does occur across species. This review is aimed at delving into species' peculiarities, exploring the diversity of venom glands and the potential ecological roles in relation to habitats and associated food webs. A detailed knowledge of the anatomy of highly diverse venom systems is a fundamental prerequisite to developing new and effective approaches to venom collection from different fish species. Here we discuss the venom collection and stabilization techniques in the light of this morphological diversity. Current extraction methods include the crushing of venomous structures, the direct extraction from glands, and pressure-based milking techniques, and the effectiveness of each technique varies depending on the species. The recent advances in venom extraction techniques, here presented, offer new perspectives not only for biotechnological applications but also to deepen into venom's broader ecological and evolutionary roles. Our review, by providing an extensive comparative characterization of the venom delivery systems in relation to fish ecology, highlight gaps that remain to be addressed. Understanding the role of sex, developmental stage, and metabolic cost of venom production in the diversification of venom delivery-structures form and function represents a key further step to promote multiple research areas and potential applicative developments.

Introduction

Recently, the remarkable potential of venom as bioactive compound has been recognised, with modern venomics showcasing its utility across diverse fields including agrochemistry, biotechnology, diagnostics, and pharmacology (reviewed in von Reumont et al., 2022). Despite the current emphasis on venom components and their promising applications in research (Bordon et al., 2020), studies on venomous fishes turn out to be poorly represented in comparison with venomous terrestrial species. Historically, detailed descriptions reported the anatomy and morphology of fish venomous spines (Allman, 1840; Borley, 1907; Byerley, 1849)**.** Together with the most recent literature dealing with molecular evolution, physiology, diversity, and omic studies on fish venom (Harris and Jenner, 2019; Smith et al., 2016; von Reumont et al., 2022), a new interest has been fuelled in this clade, especially for the biomedical and biotechnological potential hidden in the largely unexplored world of fish venom diversity. The revised estimate of venomous fishes (ray-finned plus cartilaginous), now includes at least 3000 species (Smith et al., 2016). Considering this as a minimum estimate, the number of venomous fish species is then comparable to venomous terrestrial vertebrates, estimated to be about 5000 species, 4600 of which are reptiles (Jenner and Undheim, 2017). However, while in venomous reptiles the emergence of venom, dating about 170 million years ago, is thought to be a single event (Fry et al., 2006; Vidal and Hedges, 2005), in fishes the emergence of a venom apparatus occurred independently 19 times, with remarkable examples of convergent evolution (Smith et al., 2016). The most widely supported theory suggests that the venom glands in fishes might have evolved from epidermal cells with the original function of producing a proteinaceous compound with protection purposes in scaleless fishes (Cameron and Endean, 1973; Maretic, 1988). The venom-delivery system appears to be structurally simple, though highly efficient: a grooved spine combined with a venomous gland. However, obtaining venom from fish presents some challenges, which can represent a limitation to extensive research. Here we provide an overview of the anatomy of venom apparatus in fishes, emphasizing the relation with some ecophysiological aspects not yet fully explored, together with a thorough systematic examination of venom extraction methodologies. By complementing the existing literature, this review aims to set the basis for more in-depth research on venomous fishes from morphological, ecological and biomedical perspectives.

Morphology and function of fish venom apparatus

Fishes use their venom primarily for defence: many venomous species have benthic to demersal lifestyles, often burying themselves in the sand (Table 1). As such, the evolution of venomous spines has been a significant adaptive innovation, increasing the evolutionary success of many clades.

Table 1

Clade	Lifestyle traits	Sting characters	Use of stings	Refs
Chimaeriformes	Deep-sea bathydemersal. Feeds mainly on bottom-living invertebrates	One serrated dorsal spine	Defensive	1 - 2
Myliobatoidei	Marine, brackish and freshwater. From benthic species buried in sandy and muddy bottoms to benthopelagic active swimmers. Feeds mainly on invertebrates	One serrated caudal spine	Defensive	1 - 2
Heterodontiforms (e.g. *H. francisci*)	Marine demersal. Feed on benthic invertebrates and fishes	Two spines in anterodorsal position	Defensive	1
Squaliforms	Marine clade from bathydemersal to pelagic-oceanic lifestyle. Feeds on a diversity of prey, ranging from jellyfish, squid, benthic invertebrates, and fishes	Two spines in anterodorsal position	Defensive	1
Monognathidae (e.g. *M. rosenblatti*)	Deep-sea bathypelagic. Diet habits not well studied	Single venomous fang	Prey immobilisation ?	2
Meiacanthus	Marine. Reef-associated. Feed on plankton, including microalgae, small crustaceans, and zooplankton	One pair of canine teeth	Defensive, intraspecific competition	3 - 4
Catfishes	Freshwater demersal clade. The clade is highly diversified in terms of lifestyle and feeding behaviour, ranging from detritus to aquatic insects and zooplankton, crustaceans and fish	Pectoral and dorsal spines with a high level of morphological diversification. Serrations and grooves vary significantly among species	Defensive	5 - 6
Toadfishes	Marine demersal clade with a sedentary lifestyle. Feeds on crustaceans, mollusks and small fishes	Dorsal and opercular spines	Defensive	7
Scomberoidini	Primarily marine with an active lifestyle. Enter estuaries and fresh water. Adults feed on fishes and crustaceans.	Dorsal and anal spines?	Defensive?	7
Gobiesocines (e.g. *A. artius*)	Marine benthic, reef-associated	Subopercular spines	Defensive	7
Uranoscopidae (e.g. *U. scaber*)	Marine demersal, usually found buried in the sand or mud. Predate mainly on fish	Cleithral spines	Defensive	8
Acanthuriformes (scats, rabbitfishes and surgeonfishes)	Marine demersal, usually found buried in the sand or mud. Predate mainly on fish	Scalpel-like caudal spines	Defensive? Intraspecific competition?	9
Trachinidae	Marine demersal. Sedentary lifestyle, resting on the bottom often buried. Feed on small invertebrates and fishes	Dorsal and opercular spines	Defensive	7
Stonefishes and wasp fishes	Marine demersal, reef-associated. Feeds on small fish or invertebrates	Dorsal and anal spines with venom gland in stonefish	Defensive	7
Lionfishes	Opportunistic generalist carnivores. Feed on fish, shrimp, crab, lobster, squid, snail and octopus	Dorsal, pectoral, and pelvic spines	Defensive. Actively preying?	7

Refs: 1, (Haddad et al., 2016); 2, (Harris and Jenner, 2019); 3, (Losey, 1975); 4, (Casewell et al., 2017); 5, (Egge and Simons, 2011); 6, (Wright, 2015); 7, (Froese and Pauly, 2023); 8, (Rizkalla and Philips, 2008); 9 (Schober and Ditrich, 1992).

In fishes, except for sea lampreys, fangblennies and deep-sea eel clades, venom apparatus is not associated with a muscular system. These spiny structures are typically located on the dorsal fins, or in similar structures from opercular, sub-opercular, or cleithral spines (Figure 1; Smith et al., 2016; Williamson et al., 1996). The number and type of spines can vary from single simple structured, to multiple serrated spines (Table 1). One of the most extreme examples is represented by the red Lionfish *Pterois volitans*, which possesses 18 venomous spines distributed across its dorsal, pelvic, and anal fins (Galloway and Porter, 2019). A pioneering work, dated back to 1840, illustrated the morphology of the opercular and dorsal spine in the Lesser weever (*Echiichthys vipera* - also referred to as *Trachinus vipera*) highlighting the presence of grooves along the edges that confer to the spine a typical "T" shape on the transverse section (Allman, 1840). The existence of a venom gland, nowadays classified as holocrine due to the secretions system, was described as a faint, whitish, transparent line, running up each side of the spine, partially lodged into the grooves (Byerley, 1849) composed by a capsule of connective tissue containing secreting large cells, arranged in radiating columns with an exceeding rich network of capillary blood vessels (Borley, 1907). The occurrence of grooved spines accommodating the venom glands and of tissue-specific cell types has been confirmed as a shared feature of venom apparatus in different species (Bertelsen, 1987; Casewell et al., 2017; Conway et al., 2014; Evans, 1924, 1916; Gopalakrishnakone and Gwee, 1993; Halstead et al., 1956, 1955; Lopes-Ferreira et al., 2014; Wright, 2015) with a high degree of diversification across venomous fish lineages (Figure 1).

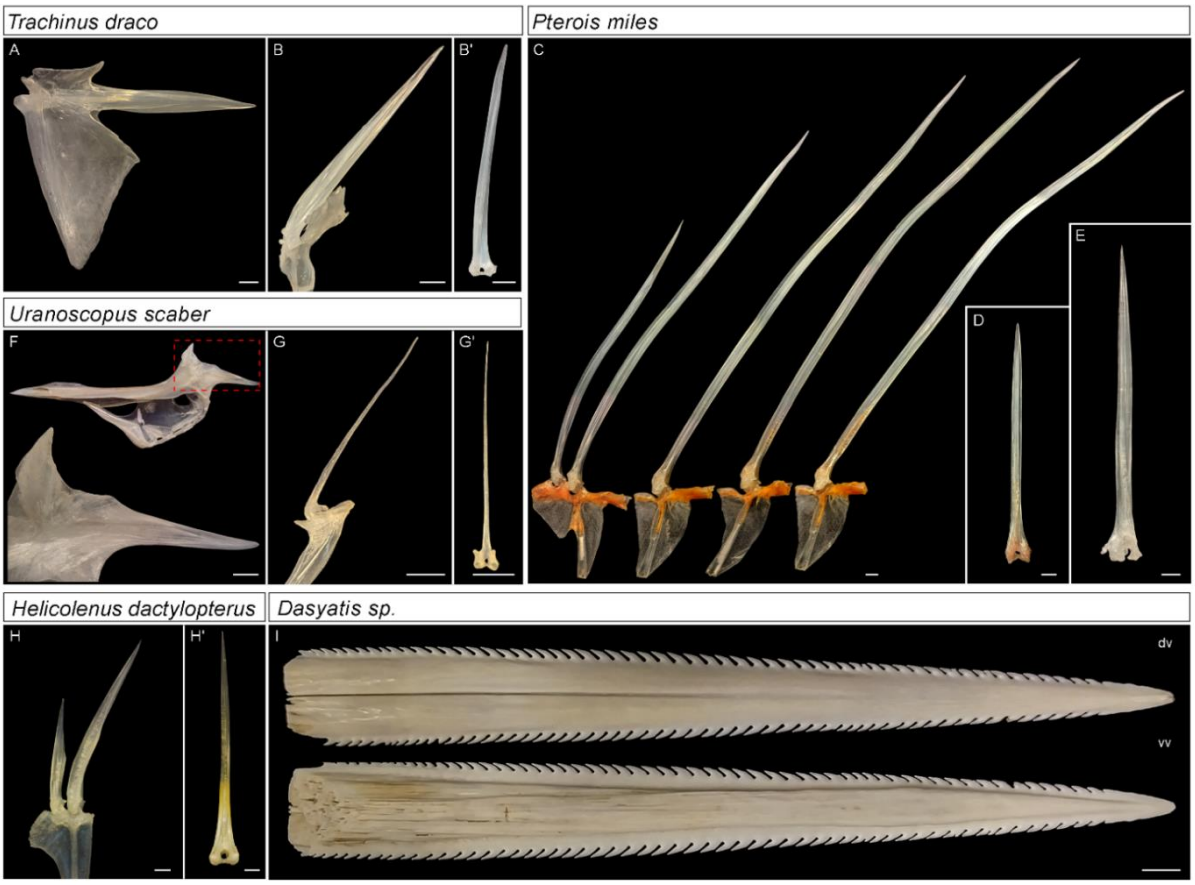

Figure 1 - Venomous spines of Mediterranean species (A, opercular; B-B', C, G-G', H-H' dorsal; D, anal; E, pectoral; F, cleithral; I, caudal). The specimens were obtained either fishery products or through scientific collaborations. dv: dorsal view; vv: ventral view. Scale bar A-H', 2mm; I, 5 mm.

Grooved spines accommodate venom glands, usually housing two venom glands in their lateral grooves, at different degrees, exhibiting a varying complexity depending on the species (Figure 2).

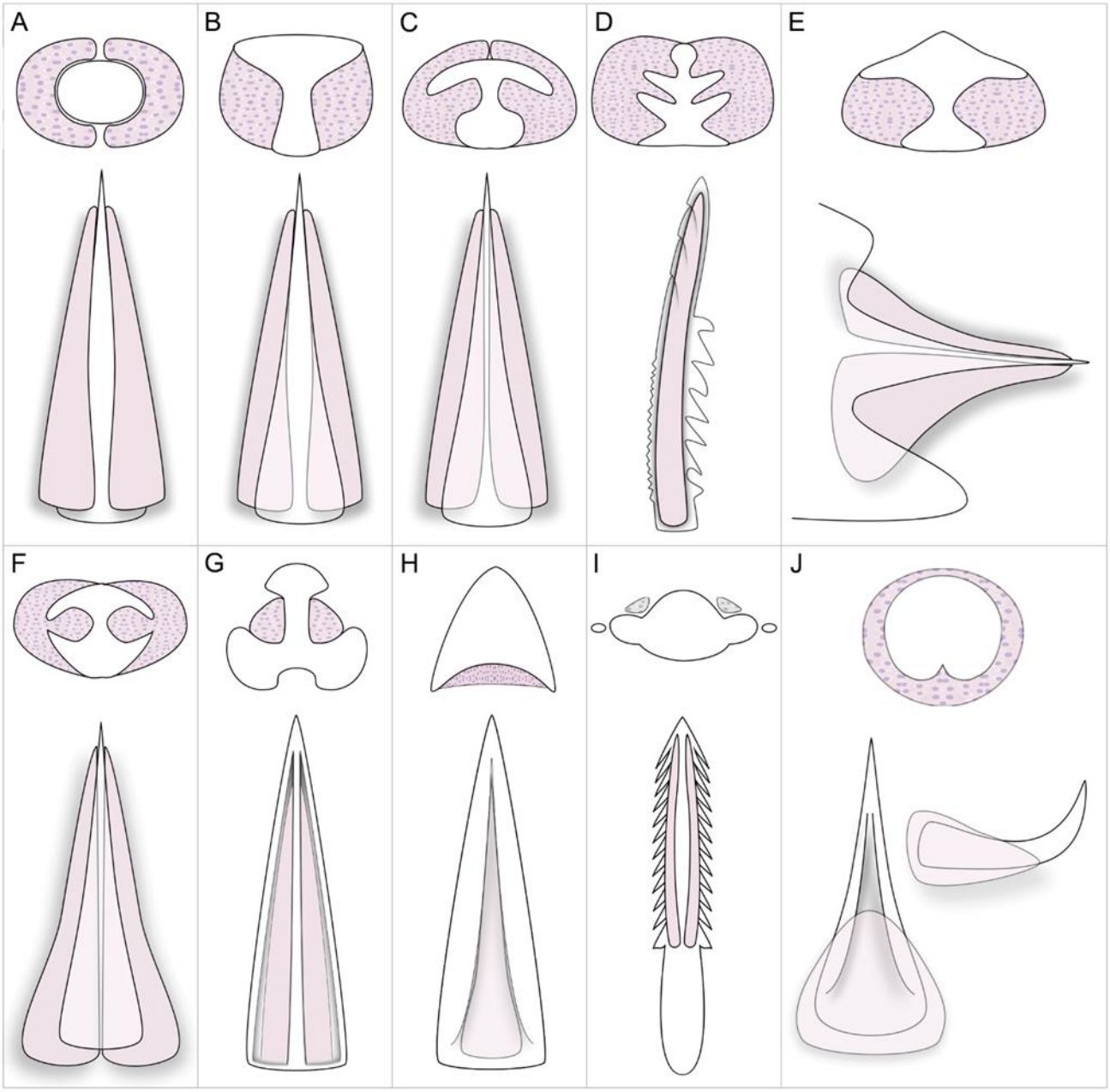

Figure 2 - Graphic representation of gland related to different venomous spines and fangs morphology. The order does not reflect any evolutionary trend. A, *Horabagrus brachysoma* pectoral spine, (Wright, 2009); B, *Acrochordonichthys rugosus* pectoral spine (Wright, 2009); C, *Noturus gyrinus* pectoral spine (Wright, 2012); D, *Noturus sp*. pectoral spine (Burr et al., 2020; Egge and Simons, 2011); E, *Trachinus draco* opercular spine (Parker, 1888); F, *Synanceja horrida* dorsal spine (Gopalakrishnakone and Gwee, 1993; Halstead et al., 1956); G, *Pterois miles* dorsal spine (Halstead et al., 1955); H, *Squalus acanthias* dorsal spine (Bargione et al., 2019; Evans, 1924)**;** I, *Dasyatis guttata* caudal spine (Pedroso et al., 2007); J, *Meiacanthus grammistes* fang (Casewell et al., 2017).

The most complete integration was observed in the stonefish *Synanceja*, where the spine grooves almost completely enclose the distal ends of the venom glands thereby transforming them into functional "ducts" (Cameron & Endean, 1972; Figure 2, F). The highest level of morphological diversification was observed in catfishes (Wright, 2015). The extent and orientation of the venom glands in relation to spine serrations, as well as grooves within the spine itself, varies significantly among species (Egge and Simons, 2011; Halstead, 1988;

Wright, 2009). Serrated spines may enhance the mechanical damage produced when the spine penetrates the skin of a potential predator and increase the surface area exposed to the concomitantly released venom (Birkhead, 1972; Egge and Simons, 2011). There is little evidence, however, suggesting that the species with such an elaborated spine shape possess significantly greater toxicity, or increased predator deterrent ability (Birkhead, 1972). Morphological adaptation, such as the loss of a venomous system, can take place when ecological pressures mutate. Accordingly, Egge and Simons (Egge and Simons, 2011) demonstrated that out of five evolutionary changes in sting morphology within the genus *Noturus*, four resulted in reduced morphological complexity up to, in one case, the complete loss of the venom gland. Such scenarios may include ontogenetic loss of venom glands in species that achieved a body size that effectively protects them from predators (Egge and Simons, 2011) or secondary loss of venom glands along with ossified fin spines loss (e.g., Malapteruridae, many amphiliids) (Wright, 2009). According to Wright (2009), members of Sisoridae and Erethistidae families have secondarily lost venom glands, while maintaining their fin spines. These families occupy fast-flowing rivers where large predatory species are nearly absent, offering a possible explanation for the loss of venom production (Wright, 2015). The occurrence of venom systems as defensive adaptations can also be transitory. In the scatophagids *Scatophagus argus* and *Selenotoca multifasciata*, the venom gland is a juvenile adaptation that disappears upon reaching adult size (Cameron and Endean, 1970). Similarly, in the acanthurid *Acanthurus triostegus* and *Prionurus microlepidotus* the presence of a venom apparatus is restricted to the larval stage (Cameron and Endean, 1973). As well as for teleosts, the morphological description of cartilaginous fish venom systems dates back to the early 1900s. Evans provided a detailed characterization of spines and venom glands in different species, depicting both Spiny Dog-Fish (*Squalus acanthias* - also referred to as *Acanthias vulgaris*) and the Sting-Ray Trygon (*Dasyatis pastinaca* - also referred to as *Trygon pastinaca*) with grooved spines filled with pearly-white glistening tissue (Evans, 1924, 1916). The few shark species that showed venom properties, belonging to *Squalus* and *Heterodontus* genera (Halstead, 1988) possess two venomous spines, located anteriorly to dorsal fins. Each spine, triangular in section, is posteriorly grooved with the groove becoming shallower towards its apex (Figure 2; Evans, 1924). In ray species, both marine and freshwater, the occurrence of a venom apparatus is more common (Haddad et al., 2016). Among them, stingrays possess one or many bony spines caudal positioned, on the dorsal surface of the tail. These stingers are dorsoventrally compressed with a sharp, pointed tip, and with lateral serrations on both sides. Two ventrolateral grooves are present along the length of the spine, housing venomous tissue covered by a protective integumentary sheath (Figures 1, 2; Shea-Vantine et al., 2021). Unlike in ray-finned fishes, stingers are associated with specialized scattered epidermal secreting venom cells rather than a proper venom gland. Interestingly, marine and freshwater species of cartilaginous fishes display a different arrangement of the venom-producing cells, which are located only in the ventrolateral grooves of the stinger in the formers, while in the latter they spread over the whole stinger epidermis (Pedroso et al., 2007). Grooved spines were also identified in fossils both in chondrichthyan (Evans, 1924) and teleosts samples (Tyler et al., 1997) including the ancestors of extant venomous fish species (Yoshinaga-Kiriake et al., 2020). This observation indicates that the occurrence of grooved spines in fossils could be a good proxy of the presence of

a venom apparatus in the lineage, and suggests that the ability to produce venom was already present in some early fish genera such as the chondrichthyan *Paleospinax*, *Sphenacanthus*, *Asteracanthus* and *Hybodus* (Evans, 1924). The number of events of venom system acquisition (followed in some cases by secondary loss) in fishes could be reevaluated by taking into account the fossil record. Little information is available on fish that acquired the venom system associated with teeth. A handful of species show these properties, including some fangblennies (genus *Meiacanthus*), deep-sea eels (genus *Monognathus*), and both blood-feeding and flesh-feeding lampreys (Baxter, 1956) (in the genera *Geotria, Mordacia* and *Petromyzon)*, highlighting contrasting characteristics in terms of the position of venom delivery structures and function of venoms. Venomous fangblenny canine teeth (or fangs) are located in the lower jaws and show anterior grooves that convey the venom produced by a large gland that surrounds the base of the fangs (Casewell et al., 2017). Venom is used by fangblennies for defence, as reported in the species *M. atrodorsalis*, which can avoid being ingested by inflicting painful bites on predators (Casewell et al., 2017) or intra- and interspecific territorial competition, as proposed by Harris and Jenner (2019)**.** On the opposite, eels seem to use envenomation for predation (Bertelsen, 1987). Studies are restricted to *Monognathus nigeli*, the only species among the 3000 known or putative venomous fish species that use fangs specifically for injecting venom during predation (Smith et al., 2016). They possess, apparently, a single tooth placed in the rostral position of the jaws, to immobilize and grasp prays, as large shrimps. However, these structures have been characterized only in leptocephalus larvae, resulting in absence in the adult stage. It has been proposed that such structures are lost during metamorphosis but still, few data are available for this elusive species (Bertelsen, 1987). Given the substantial overlap in protein composition and function between hematophagous secretions and traditional venoms, hematophagous animals, including lampreys, are considered venomous (Fry et al., 2009). The presence in lampreys of a series of epidermal teeth connected to a venomous gland, through which they release bioactive secretions containing anticoagulants, ion channel blockers, and immune suppressors, support their classification as venomous animals (Gou et al., 2022). The morphology and location of buccal glands vary among lamprey families. In *Geotria australis*, they are bean-shaped sacs embedded in the basilaris muscle, lined with columnar epithelium and surrounded by skeletal muscle (Cook et al., 1990). *Mordacia mordax* has two lateral and one central buccal gland, containing ductless secretory units that discharge directly into the buccal cavity (Potter et al., 1995). In *Petromyzon marinus*, the glands are thin-walled sacs located ventrally in the basilaris muscles (Gibbs, 1956). The extreme variability in venom systems among venomous fishes calls for further investigations to enhance our understanding and improve methods for venom collection across different species. These systems, primarily evolved for defense, showcase remarkable morphological adaptations and developmental patterns influenced by ecological pressures. Investigating venom system architectures will provide insights not only into the evolutionary success of these fishes but also into potential applications for biomedical research and toxin development.

Fish venom collection

Main methodologies for venom stabilization and conservation were recently reviewed (Gorman et al., 2020) but, among several experimental difficulties, including the presence of mucus, the transparency and small size

of the glands, together with the difficult accessibility inside the grooved spines, venom collection still represents a critical issue. Venoms are composed of a cocktail of toxins, enzymes, bioactive peptides and non-proteinaceous components (Gorman et al., 2020). It was reported that extraction methods can influence the product composition, in several organisms including spiders (Lyons et al., 2023), scorpions (Ozkan et al., 2007), ants (Aili et al., 2017), wasps (Mueller et al., 1981) and jellyfish (Cantoni et al., 2020). Over the years, researchers adopted several methods to collect fish venom as a pure extract or collected the entire venomous apparatus (Table 2). For *post-mortem* venom extraction, the earliest procedures involved crushing the venomous spines and adjacent tissues in a physiological saline solution or glycerine (Chhatwal and Dreyer, 1992). In alternative, the gland was macerated in a solution of water with chloroform-glycerine. The resulting fluid, crude or filtered, was then used for downstream analyses (reviewed in Evans, 1907). Approaches employed in more recent works were similar. The spines were manually extracted, homogenised, and macerated with liquid nitrogen. After adding water, the solution was centrifuged and the supernatant was lyophilized and stored at -20 °C until use (Mohamadi et al., 2015; Sarmiento et al., 2015)**.** Alternative procedures involve the sonication of the venom apparatus, the collection of the supernatant after centrifugation, a dialysis step against MilliQ water, and filtration. The solution obtained is lyophilized and stored at −20 °C until further use (Fezai et al., 2016). The use of freshly caught specimens was described as well. A simple venom milking method includes the application of mechanical pressure onto the spines, forcing them to draw off the released liquid from the tip. Such methodology resulted effective only for some fish species, such as toadfish or stonefish (Sosa-Rosales et al., 2005; Ziegman et al., 2019). To obtain pure venom extract, different techniques involve a direct extraction from the venom-producing gland, using a sterilized syringe by inserting the needle in the groove of the spine (Evans, 1907; Harris et al., 2021) or a vacuum suction apparatus where a rubber tube fits with the venomous spine (Skeie, 1962). More complex techniques are used for living specimens, allowing for multiple milking events. In one of these experimental procedures, the fish was kept in small tanks and a pressure was applied on the spines with a small fragment of expanded polyurethane sponge held in forceps. Once adsorbed the mixture, the sponge was rinsed out in distilled water and squeezed (Carlisle, 1962). A more recent version of this protocol involves the use of a synthetic sponge placed into a collecting tube. The sponge is pressed against the venomous spine absorbing the venom, reducing losses or contaminations (Almada et al., 2016). A similar approach was adopted for venomous bite fish, replacing the sponge with a dry cotton swab. After the bite, the swab was vortexed in 50% acetonitrile and centrifuged. The supernatant was filtered and the relative protein concentration was measured with a nanodrop spectrophotometer (Casewell et al., 2017). In cartilaginous fish, the absence of a proper venom gland (Diaz, 2008; Pedroso et al., 2007) makes milking impossible, and the spine with attached the venom-secreting tissue is difficult to crush. A recent publication reported a crude venom extraction obtained by scraping the tissue from the tail spines of live specimens and using a methanol-based extraction protocol. The sample was then frozen and powdered in liquid nitrogen and a mortar. The powder was then dissolved in methanol and subjected to incubation at various temperatures. The next day the mixture was centrifuged, and the resulting supernatant was therefore collected and lyophilized (Kirchhoff et al., 2021). Previous studies reported that, in most fish

groups, both the venom gland and delivery system can be identified under a dissecting microscope (Halstead, 1988). For some species, it could be theoretically possible to dissect the gland on ex vivo or anesthetized specimens, but the consistency, size, transparency of the gland and its secretion together with the epidermal tissue make this practice difficult execution. To the best of our knowledge such venom gland sampling was reported for lampreys only (Li et al., 2018)**.** The summarized reports highlight the heterogeneity in venom extraction methods employed across fish species. However, a broad-scale comparative study through fish venom systems is essential to properly evaluate the efficiency of venom milking techniques. While recent work has reviewed methodologies for venom stabilization and conservation (Gorman et al., 2020), challenges remain, including mucus interference, gland accessibility, and method-induced variations in venom composition. Addressing these issues will enhance our understanding, allowing both basic and applied research in fish venom research.

Table 2

	Extraction Method	Specie	Application	Refs
Post-mortem procedures	Crushed venom structures in a physiological saline solution or glycerine	*Trachinus draco*	Toxicity test Haemolytic activity	1
	Macerated gland in a solution of water with chloroform-glycerine		Toxicity test Blood pressure Respiratory system assessment	2
	Spines manually extracted, homogenised, and macerated with liquid nitrogen	*Scatophagus argus argus*	Anatomical features	3
		Pimelodus maculatus	Nociceptive behaviour Haemolytic activity Cardiotoxic activity	4
	Venom apparatus was cutted, crushed and sonicated in	*Echiichthys vipera*	Cell viability test Cancer cell suppression	5
Freshly-caught Specimens	Mechanical pressure onto the spines	*Synanceia horrida*	Venom composition	6
		Thalassophryne maculosa		7
	Inserting a syringe directly into the venom gland	*Trachinus draco*	Toxicity test	2
		Synanceia verrucosa	Coagulotoxic effects Neurotoxic effects	8
	Vacuum suction apparatus	*Trachinus draco*	Toxicity test Venom stability	9
	Stinging the fish with a polyurethane sponge	*Echiichthys vipera*	Anticoagulant activity Lethal Dose assessment	10-11
	Venomous fish biting a dry cotton swab	*Meiacanthus grammistes*	Venom composition	12
	Frozen and powdered in liquid nitrogen scraped tissue from the tail spines	*Dasyatis pastinaca* *Himantura leoparda* *Pteroplatytrygon violacea* *Potamotrygon leopoldi* *Potamotrygon motoro*	Venom tissue Transcriptome Venom Bioactivity	13
	Buccal glands dissection and venom collection with a syringe	*Lampetra morii*	Proteomic analysis of buccal gland secretion	14

Refs: 1, (Chhatwal and Dreyer, 1992); 2, (Evans, 1907); 3, (Mohamadi et al., 2015); 4, (Sarmiento et al., 2015); 5, (Fezai et al., 2016); 6, (Ziegman et al., 2019); 7, (Sosa-Rosales et al., 2005); 8, (Harris et al., 2021); 9, (Skeie, 1962); 10, (Carlisle, 1962); 11, (Almada et al., 2016); 12, (Casewell et al., 2017); 13, ((Kirchhoff et al., 2021); 14, (Li et al., 2018).

Conclusion

The evolutionary history of venom systems in fish highlights that, while a backbone structure is conserved among the clades, multiple significant morphological differences characterize the delivery system and gland morphology. This complicates the development of a universal venom extraction method. A deep understanding of venomous anatomy across species is therefore essential. Although research on fish venom is limited to a few species, recent advances are uncovering venom's biological diversity and potential applications in various fields. These considerations emphasize the importance of refining techniques to ensure effective collection without compromising the integrity of the venom and venomous structures.

Moreover, understanding the ecological contexts in which these venoms are employed, whether for defence, predation, or competition, offers deeper insight into their evolutionary significance. Key areas for future explorations include a study of intraspecific venom variability, especially with larval stages' diverse ecologies and gender-specific or behavioural differences, the study of the nektonic clades, such as scats, rabbitfishes, and surgeonfishes, the incorporation of fossil records. Examination of sexual dimorphism in venom composition and morphology of venom delivery structures is, for example, still very limited to a few fish species such as the Cano toadfish and the Atlantic stingray (Harris, 2023). Knowledge of the metabolic cost imposed by venom delivery systems remains largely untapped. The sole study attempting to quantify the metabolic investment in spine structure and maintenance in a fish, the Atlantic stingray (Enzor et al., 2011), indicates that the total average cost represents approximately 0.04% of the fish's daily metabolic expenditure. It is known, though, that in other venomous organisms the production imposes a much higher metabolic cost. Pit vipers can elevate their daily caloric budget by around 11% (McCue, 2006), while the metabolic expenditures in the South African fat-tailed scorpion can amount to as much as 39% (Nisani et al., 2007). The stingray venom apparatus lacks a distinct venom gland and indeed cannot be considered a typical fish venom system (Pedroso et al., 2007), leaving the question open. The metabolic cost of defensive venom production and its evolutionary implications on the evolution of fish venom systems are a research field worth of further exploration.

In conclusion, the call for a more comprehensive characterization of fishes' venomous species echoes in the realms of biology, ecology, zoology, physiology, and evolutionary studies, promising a deeper understanding of these diverse groups of animals.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

GA and **AT** conceived the project, reviewed and summarized the primary literature, and wrote the original draft. **GA**, **AT**, **LL**, **PDL** and **MVM** reviewed and edited the manuscript. All authors approved the submitted version.

Data availability statement

No new data are produced in this study. Data sharing is not applicable to this article.

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