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Chemical and physical defenses against predators in *Cystodytes* (Ascidiacea)

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Abstract

Ascidians utilize both physical (spicules, tunic toughness) and chemical defenses (secondary metabolites, acidity) and suffer relatively little predation by generalist predators. The genus *Cystodytes* (Polycitoridae) is distributed widely in both tropical and temperate waters. Secondary metabolite composition, calcareous spicules and tunic acidity ($pH<1$) may act as redundant defense mechanisms against predation in this genus. To assess the relative importance of chemical and physical defenses against predation in ascidians, we studied purple and blue morphs of *Cystodytes* from the western Mediterranean (formerly assigned to *Cystodytes dellechiajei*, but recently shown to belong to two different species), and a purple morph from Guam (USA), identified as *Cystodytes violatinctus*. Crude extracts, spicules, ascididemin (the major alkaloid of the blue morph) and acidity were used in feeding trials to evaluate chemical and physical defense mechanisms in *Cystodytes* spp. We performed feeding experiments in the field with a guild of generalist fish (mostly damselfish), and in the laboratory with a sea urchin and a puffer fish. Our results showed that all crude extracts and ascididemin significantly deterred fish predation, but not sea urchin predation. However, neither acidity alone nor spicules at natural concentrations deterred feeding. These results and other studies on sponges and gorgonians suggest that secondary metabolites are the primary means of defense against fish predators. Spicules and tunic acidity may perform other ecological roles and/or target certain specialist predators.

Keywords: Acidity; Ascidians; Ascididemin; *Cystodytes*; Defense mechanisms; Deterrence; Predation; Toxicity

1. Introduction

Benthic marine invertebrates are under intense competitive pressure for space, light and nutrients. In addition, sessile organisms are easy prey for predators. Many of these organisms, therefore, have developed a range of defense mechanisms, including behavioral (e.g. mimicry), physical (e.g. spicules, tissue toughness) and chemical (e.g. bioactive secondary metabolites, acid pH) strategies, to ensure survival. The relative importance and potential interactions of different defense mechanisms may depend on: the organism examined (Pennings and Paul, 1992; Hay et al., 1994), the inability of a single defense to deter all type of predators and/or competitors (Paul and Hay, 1986; Hay et al., 1987; Schupp and Paul, 1994; Pisut and Pawlik, 2002; Tarjuelo et al., 2002; Burns et al., 2003), the

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organism's life history stage (Uriz et al., 1996; Pisut and Pawlik, 2002), the developmental or physiological constraints that might prevent a particular defense from being used in all parts of the organism, necessitating additional defenses to ensure protection of the whole organism (Harvell and Fenical, 1989), evolutionarily established constraints (Schmitt et al., 1995; Kubanek et al., 2002) and whether structures or compounds are energetically expensive, among other factors. For instance, in some sponges, both large spicules and chemical defenses deter fish feeding (Pawlik et al., 1995; O'Neal and Pawlik, 2002; Burns et al., 2003; Burns and Ilan, 2003). Soft corals and gorgonians produce both chemical defenses and sclerites, which, in some cases, protect them against predators (Pawlik et al., 1987; Harvell et al., 1988; Wylie and Paul, 1989; Pawlik and Fenical, 1992; Puglisi et al., 2000, 2002; O'Neal and Pawlik, 2002). However, the primary function of sclerites may be structural rather than defensive (Koehl, 1982; Lewis and Von Wallis, 1991). In view of these and similar results, Van Alstyne et al. (1994) suggested that, when feeding experiments are designed, all defense mechanisms against predation that an organism might use should be tested in combination, rather than each single defense separately. However, mainly due to experimental limitations, few studies have attempted this (Paul and Van Alstyne, 1992; Schupp and Paul, 1994; O'Neal and Pawlik, 2002; Hill et al., 2005).

Among benthic invertebrates, ascidians suffer relatively little predation by generalist predators (Millar, 1971; Goodbody and Gibson, 1974; Stoecker, 1980a). These generalist predators are mainly fish (Randall and Hartman, 1968; Myers, 1983), and occasionally urchins (Briscoe and Sebens, 1988). Specialist predators on ascidians include mollusks, such as the lamellarians, cypraeids (Fretter and Graham, 1962; Millar, 1971; Lambert, 1980) and nudibranchs (Millar, 1971; Paul et al., 1990), and polyclad flatworms (Millar, 1971; Morris et al., 1980; Schupp et al., 1999; Caralt et al., 2002).

In addition, ascidians have both physical (spicules, tunic toughness) and chemical defenses (Swinehart et al., 1974; Stoecker, 1978, 1980b; Pisut and Pawlik, 2002; Tarjuelo et al., 2002). Numerous studies support the deterrent role of secondary metabolites (Paul et al., 1990; Davis, 1991; McClintock et al., 1991; Lindquist et al., 1992; Vervoort et al., 1998). High vanadium concentrations (Stoecker, 1980b) and low pH (Webb, 1939; Stoecker, 1978, 1980a,b,c; Pisut and Pawlik, 2002) have also been suggested as anti-predatory defenses. However, other studies indicated that neither the presence of vanadium nor an acidic pH prevented predation (Parry, 1984; Tarjuelo et al., 2002). All in all, little experimental evidence exists regarding the interaction of chemical and physical defense mechanisms in ascidians.

We chose the widely distributed ascidian genus *Cystodytes* (Aplousobranchiata: Polycitoridae) to assess the relative importance of physical and chemical defenses against predation in ascidians. López-Legentil et al. (2005a) identified two chemotypes within Mediterranean specimens of this genus, previously attributed to *Cystodytes dellechiajei* (Della Valle, 1877). Genetic and biological evidence indicates that these two chemotypes correspond to sibling species (López-Legentil and Turon, 2005; López-Legentil et al., 2005b). The first chemotype was characterized by the presence of C_9-unsubstituted pyridoacridines such as ascididemin (Fig. 1; Kobayashi et al., 1988) and 11-hydroxyascididemin (Schmitz et al., 1991), which were present in blue and green morphs from the western Mediterranean. The second chemotype contained the sulfur-containing pyridoacridines kuanoniamine D (Caroll and Scheuer, 1990), shermilamine B (Carroll et al., 1989) and their deacetylated forms (Eder et al., 1998; and López-Legentil et al., 2005a). These pyridoacridines were present in a purple morph from the western Mediterranean, and were also found in a purple morph from Guam, identified as *Cystodytes violatinctus* Monniot, 1988 (López-Legentil, 2005). Some of these substances are highly cytotoxic (Eder et al., 1998; Dassonneville et al., 2000; Bowden, 2000). Ascididemin also has some antibacterial and antifungal action (Lindsay et al., 1995). To our knowledge, apart from a possible antifouling function (Debard et al., 1998), no ecological role has been described for ascididemin. In addition to the bioactive compounds cited above, *Cystodytes* spp. colonies are highly acidic (pH < 2; Parry, 1984; Tarjuelo et al., 2002) and contain calcareous spicules encasing the zooids (Turon, 1987; Kott, 1990). These potential defenses seem to result in an effective defense mecha-

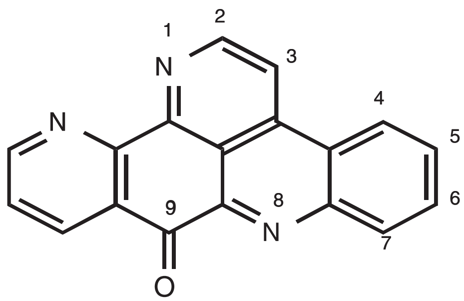

Fig. 1. Chemical structure of ascididemin, the major alkaloid of the Mediterranean blue morph.