

## On the taxonomic status of the brittle-star *Amphipholis squamata* (DELLE CHIAJE, 1828)

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### Summary

*Amphipholis squamata* is one of the 15 species of echinoderms of the Belgian fauna. Recent works on the intraspecific variability suggest that this species might constitute a complex of sympatric colour morphs. This short paper aims to review the research on this field and to clarify the taxonomic status of this brittle-star.

### Résumé

*Amphipholis squamata* est une des 15 espèces d'échinodermes de la faune belge. Des travaux récents sur la variabilité intraspécifique suggèrent que cette espèce pourrait constituer un complexe d'espèces surs vivant en sympatrie. L'objectif de cet article est de synthétiser les recherches réalisées dans ce domaine et de clarifier le statut taxonomique de cette ophiure.

### Introduction

The species is the lowest level of classification. In sexually-reproducing organisms, it is a group of individuals that can usually or potentially interbreed and which are reproductively isolated from other such groups [3, 29, 30, 31]. After centuries of species descriptions, numerous mistakes were discovered. Most of the time, a species was described several times under different names because of its polymorphism or its large geographical distribution. For example, the red seaweeds *Porphyra umbilicalis* and *Conchocelis rosea* are very common on the coast of Europe. They have contrasting morphology since *Porphyra* have the form of an upright sheet or leaf whereas *Conchocelis* is a branch, filamentous seaweed which is able to bore into the calcareous shells of molluscs and barnacles. Carpospores of *Porphyra* were cultured in laboratory and it quickly became clear that the filamentous algae produced were identical with *Conchocelis* [41]. Numerous mistakes were then corrected and the quantity of known species decreases with the time, constituting a virtual biodiversity crisis. Fortunately, a new taxonomy appeared with modern biology. New tools such as genetic markers induced modifications in the classification of the taxons. New criterions were then

used to separate species: enzyme activity, genetic distances, etc. [29].

The small ophiuroid species *Amphipholis squamata* (DELLE CHIAJE, 1828) is one of the 15 echinoderm species of the Belgian fauna. It is a good illustration of the difficulty to design correctly a taxon and it constitutes an interesting model to discuss the validity of the taxonomical parameters (genetic, physiology, morphology, etc.).

### *Amphipholis squamata*

*A. squamata* is probably "the most widely mentioned *Amphiurid brittle-star in scientific literature*" [28, 39]. Various aspects of its biology have been studied in several areas of the world since the 19<sup>th</sup> century. First known description was published in 1805 by VIVIANI [42] who described the luminous capabilities of the ophiuroid. Since this period, numerous papers appeared regularly in the literature but for fifty years, the frequency of publications increased exponentially. The unexpected interest for this small ophiuroid could be explained by its amazing biological properties: *A. squamata* is cosmopolitan [21, 22, 26], bioluminescent [4, 42], polychromatic [2, 11, 12, 13, 14, 16, 17, 18, 19, 20, 40], hermaphrodite, brood protecting [1, 21]; both selfing and outcrossing occur for reproduction [18, 20, 36], etc. *A. squamata* is believed to be the only ophiuroid with a world-wide distribution excepted in polar regions [21, 22, 26]. As a consequence of this distribution, the species appeared in the literature under a variety of generic and specific names over the last two hundred years and more than thirty synonyms were proposed [1, 5]. Surprisingly, global populations of *A. squamata* are morphologically uniform [21, 24, 38]. It appeared to vary but little between distant populations. For example, no differences were observed in morphology between animals from Britain and New Zealand [21]. Reason why CLARK underlined the importance to stabilize the specific name and the name *Amphipholis squamata* (DELLE CHIAJE, 1828) was formalized by the International Commission on Zoological Nomenclature in 1987 [5].

### A complex of species?

Recent works on eco-physiology of *A. squamata* report loose inter- and intrapopulation variability at all levels from population to genotype.

(i) The species displays various colours of disc and arms. Eleven colour morphs were described in different stations around the world [2, 11, 12, 13, 14, 16, 17, 18, 19, 20, 40]. Since adult body colour remains constant through time and since juveniles present always the same body colour than its parent, BINAUX & BOCQUET suggested that colour morphs are genetically determined and that most of the reproduction occurs by selfing [2].

(ii) The species is bioluminescent. It emits visible light when mechanically stimulated. The nervous control of the light emission was studied and several differences in the physiology of luminescence were observed between colour morphs [6, 7, 8, 9, 10, 27]. Recently, it has been demonstrated that each colour morph possesses its own luminous capabilities [11, 12, 13, 14, 16, 17, 18, 19]. In laboratory conditions, DEHEYN *et al.* [11] have confirmed the results of BINAUX & BOCQUET on inheritance of body colour and they have shown that luminous capabilities are also transmitted to the offspring even in presence of individuals of other colour morphs. They suggest that cross-fertilization can only happen between individuals of the same variety. On the basis of these observations, some authors suggest, “*The ‘colour-luminescence’ link appeared clearly fixed (...) and is suggested to be of genetic origin. The species ‘A. squamata’ may then be a mosaic of genetically different entities (the varieties) rather than a unique cosmopolitan taxonomic entity*” [14] and “*ophiuroid colour varieties are reproductively isolated sibling species*” [11].

(iii) Differences between the colour morphs were also observed for life-history traits. We observed two colour morphs, co-existing in the same population, with different reproductive period (one in Autumn and another one mainly in Spring). This observation suggests that colour morphs are nearly reproductively isolated and that sympatric speciation could occur [15, 16].

(iv) In 1989, MURRAY has performed the first genetic analysis on this species. He has demonstrated that the species genetic identity is low (31 %) between individuals from UK and USA with a high genetic polymorphism that can reach 14 different alleles for some loci [33]. Another study of the genetic diversity at high geographical levels was realized by SPONER *et al.* [38]. Using mitochondrial DNA sequencing they revealed high genetic differences among populations since they observed deep divergence of up to 23.4 % which is ten times higher than reported levels of intraspecific divergence in other echinoderms. On the basis of these observations, they proposed: “*divergent clades are likely to represent different species*”. Genetic variation was assessed at smaller geographic scale by POULAIN *et al.* [36]. Using RAPDs markers, they have demonstrated that both selfing and outcrossing were used for reproduction in *A. squamata*. Moreover, they observed a strong genetic diversity, even in the same

sampling site. We used the same method in order to demonstrate that body colour and luminous capabilities are genetically determined. Genetic structure is homogenous for each colour morph within a population while it shows inter-population variations for a same variety [19].

### One species?

*A. squamata* is then a complex of sympatric colour morphs. Within a population, these morphs are genetically determined and huge differences between them were observed in their life-history traits and bioluminescence properties. Two hypotheses arise: (a) the “*sibling species hypothesis*”: this ophiuroid represents different species living in sympatry or (b) the “*one species hypothesis*”: it is a single highly variable species, which “*harbours unprecedented levels of genetic divergence*” [37]. DEHEYN *et al.* [11, 14], working on bioluminescence and polychromatism, and SPONER *et al.* [38], working on genetic macro-variations, support the “*sibling species hypothesis*”. Nevertheless, other works on morphology and reproduction of the species give useful information supporting the “*one species hypothesis*”.

(i) If the species have been reproductively isolated for millions of years, as expected by the huge genetic differences, some fixed morphological differences are expected between individuals. Using scanning electron microscopy, SPONER *et al.* [38] have carried out a morphometric study of the micro-morphology of *A. squamata* specimens of contrasting genotype. Surprisingly, only few differences were observed between them.

(ii) *Amphipholis squamata* is a simultaneous hermaphrodite [1, 21, 25, 35]. Both testis and ovary are paired in the same genital bursae and reach maturity simultaneously [1, 34]. Because of this anatomy, the species was suspected of self-fertilization [34] and/or of parthenogenesis [4, 32]. More recently, the possibility of selfing was demonstrated both in laboratory [11] and natural [18, 20, 36] conditions. POULIN *et al.* [36] have also demonstrated that outcrossing could occur. Using data on inheritance of body colour and luminous capabilities in laboratory conditions, DEHEYN *et al.* [11] suggested that: “*If cross-fertilization could indeed normally occur (...) it was not successful between the beige and the black variety (...) It is possible that cross-fertilization is only successful between individuals of the same variety*”. Using a similar method, we have showed that outcrossing between different colour morphs could occur in natural conditions since we have observed juveniles with luminous capabilities different from their parent [18, 20]. Only few data are available on the frequency of the monoparental reproduction. Some authors suggest that selfing might be the main way of reproduction [2] when others affirm that it constitutes only an alternative way of reproduction [11]. POULIN *et al.* [36] said: “*selfing and outcrossing rates probably depend on the spatial distribution of local population and the type of habitat*”.

## Conclusion

*Amphipholis squamata* has a worldwide distribution being absent only in polar region. It occupies a wide range of habitats from intertidal zone down to 1330 m depth [21, 22, 26]. It is constituted of dense local populations in various biotopes: under stones of tide pools, in phanerogam meadows (*Zoostera*, *Cymodocea*, etc.), in seaweeds (*Chaetomorpha*, *Corallina*, etc.), endosymbiotic of sponges, in coral reefs, etc. In 1987, CLARK proposed to stabilize the name *Amphipholis squamata* and to suppress synonyms [5]. Since this period, numerous works were performed in various fields. They showed that an important intraspecific variability is the rule at all the studied levels, from gene to populations. Moreover, this variability is linked to the polychromatism. For example, within a population each colour morph possesses its own luminous capabilities, which are genetically determined [19]. On the basis of these observations, several authors suggest that *A. squamata* represents different sibling species, each colour morph being a new species [11, 14, 18]. Moreover, genetic structure shows important inter-population variation for the same colour morph [19]. On the basis of these criteria, *A. squamata* must be divided in dozens species. On the other hand, global populations of *A. squamata* are morphologically uniform and no fixed morphological differences were observed between individuals with contrasting genotypes [21, 24, 37]. Moreover, we have demonstrated that outcrossing is possible between colour morphs [18, 20]. Therefore, on the basis of the biological definition of the species, we propose to consider *Amphipholis squamata* as a unique species. This species "harbours unprecedented levels of genetic divergence" [37] inducing a high level of intraspecific variability. This variability is probably the clue to explain its incredible ecological success.

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