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# One or many? Molecular versus morphological diversity in the aplacophoran *Chaetoderma nitidulum* Lovén, 1844 (Mollusca: Caudofoveata)

Nina T. Mikkelsen[1,2] and Christiane Todt[1,3]

[1]*University Museum of Bergen, University of Bergen, 5020 Bergen, Norway;*
[2]*Department of Biology, University of Bergen, 5020 Bergen, Norway; and*
[3]*Rådgivende Biologer AS, Bredsgården, Bryggen, 5003 Bergen, Norway*

*Correspondence: N.T. Mikkelsen; e-mail: nina.mikkelsen@um.uib.no*

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## ABSTRACT

*Chaetoderma nitidulum* Lovén, 1844 is a common species of caudofoveate (chaetodermomorph) with a wide distribution range in the northeastern Atlantic Ocean. It was the first species of aplacophoran mollusc to be described, but its species identity has been debated. Here, we investigate the molecular and morphological diversity of specimens from a large geographical area and size range, which had been preliminarily identified, based on morphology, as *C. nitidulum*. Analyses of molecular data revealed two distinct genetic sister lineages in the Eastern Atlantic and one clade sister to these in the Western Atlantic. Morphological analyses showed significant variation that does not reflect the genetic linages. In addition, molecular phylogenetics combined with comparative morphological analyses showed that radula characters used to distinguish the genera *Chaetoderma* and *Falcidens* within Chaetodermatidae do not represent apomorphies, but are a result of ontogenetic changes in *C. nitidulum*. The emerging phylogenetic patterns were surprising, bringing us one step closer to understanding chaetodermatid ontogeny and evolution, but they also emphasize that the systematic relationships of chaetodermatid caudofoveates are still far from understood.

## INTRODUCTION

Caudofoveata, also known as Chaetodermomorpha, is a taxon of shell-less, vermiform molluscs burrowing in marine soft sediments. The first species of Caudofoveata to be described was *Chaetoderma nitidulum* (Lovén, 1844). This relatively large-bodied species measures up to 8 cm in body length and has a distribution range from the Svalbard Archipelago in the north to the British Isles in the south (Salvini-Plawen, 1975). In Scandinavian waters it is commonly found from about 30 m to 400 m depth, but there are some records exceeding 1,000 m depth (unpublished data).

The original description of *C. nitidulum* from the Swedish west coast consists of a very brief account in Latin, complemented by a drawing of the animal and a few of its sclerites (Lovén, 1844). Lovén himself did not assign any type material, but later a jar containing four specimens of caudofoveates supposedly collected by Lovén from a site close to the type locality and labelled "*Chaetoderma nitidulum*" and "types" was deposited in the Swedish Museum of Natural History (Stockholm; Fig. 1). This type series, however, contained one specimen of a different species, *Falcidens crossotus* (Salvini-Plawen, 1968), which was later separated from the lot by Salvini-Plawen. Based on the slightly different morphologies of the three remaining specimens, Ivanov & Scheltema (2000) suggested that even more species were represented in the type material. The specimen illustrated by Lovén in the original description (1844) does not closely resemble any of the specimens in this museum lot and it is doubtful whether it is included.

An animal similar to *C. nitidulum* was later described under the name *Crystallophrisson nitens* (Möbius, 1875). Around the same time, Théel (1875) introduced the family Chaetodermidae, which was later changed to Chaetodermatidae (Marion, 1885). Shortly afterwards, more detailed anatomical descriptions of *C. nitidulum* were published by von Graff (1876), Hansen (1877) and Wirén (1892a). It was also concluded that the brief description of *Crystallophrisson nitens* by Möbius (1875) referred to *C. nitidulum* and the names were consequently synonymized (Graff, 1876; Lütken, 1877; Wirén, 1892a; Salvini-Plawen, 1984). Some decades later, however, Thiele adopted the genus name *Crystallophrisson* and changed the family name to Crystallophrissonidae (Thiele, 1932), the genus name *Chaetoderma* having been considered invalid because it was preoccupied by a genus of fish. This decision was later renounced (Opinion 764, International Commission on Zoological Nomenclature, 1966) and since then *Chaetoderma* and Chaetodermatidae have been valid names.

Today, Chaetodermatidae is one of three recognized families within Caudofoveata, along with Prochaetodermatidae and Limifossoridae. Chaetodermatidae is the most species rich of the three families, with 76 described species. The overall morphology within the genus *Chaetoderma* is quite uniform. Species are hardly distinguishable to the untrained eye and even experts find the application of species-level characters for identification to be challenging.

Morphological characters used for classification and identification of caudofoveate species are the shape and proportions of the


**Figure 1.** Syntypes of *Chaetoderma nitidulum* (Lovén, 1844) (lot 1422, Naturhistoriska Riksmuseet, Sweden).

body, the shape of the oral shield that surrounds the mouth opening, and the morphology of hard parts, i.e. the epidermal sclerites covering the body and the radula. Sclerite morphology in caudofoveates varies among different body regions, the anterium, neck, trunk and posterium (Schander, Scheltema & Ivanov, 2006). For species identification, it is usually necessary to make microscopic preparations of sclerites and radula.

The main defining character of Chaetodermatidae as a taxon is a radula reduced to a single pair of denticles attached to a cone-shaped structure (Salvini-Plawen, 1968). The only diagnostic characters separating genera within the taxon are also connected with radula morphology (Salvini-Plawen, 1968; Scheltema, 1972). In *Falcidens*, the radula consists of a pair of sickle-like median teeth connected by a proximal symphysis and attached to the cone, and a central plate with two apophyses that wrap around the teeth. The radula of *Chaetoderma* is defined as having a pair of smaller denticles sitting on lateral projections connected with the radular membrane, which forms a dome that completely surrounds the radula (Scheltema, 1972; Salvini-Plawen, 1975; Ivanov, 1979). In the monotypic *Furcillidens*, the radula morphology diverges from that of the other genera in the family in completely lacking denticles; instead, the radula has a forked projection that supports the cuticular dome (Scheltema, 1998). In addition, Ivanov (1981) described the radula of *Caudofoveatus*, another small genus with two species, to be more complex than that of *Chaetoderma*, including a plate attached to the cone, which holds two small additional denticles, resulting in a radula with four denticles in total. Salvini-Plawen (1984) argued that the differences in radula morphology are not indicative of separate genera and included the representatives of this genus in *Chaetoderma*, based on similarity of their sclerites.

Within the genus *Chaetoderma*, 47 species are currently recognized (World Register of Marine Species, 2017). Apart from *C. nitidulum*, two other species of *Chaetoderma* have been described from the northeastern Atlantic: *C. productum* (Wirén, 1892b) and *C. intermedium* (Knipowitsch, 1896). The latter was originally described as a subspecies of *C. nitidulum* and has been regarded as such by some authors (e.g. Thiele, 1932). *Chaetoderma productum* has likewise been regarded as a subspecies of *C. nitidulum* by some authors (e.g. Odhner, 1921). Both *C. intermedium* and *C. productum* have a more northern distribution (Arctic Ocean from the Kara Sea to Greenland) than *C. nitidulum*, but their distributions do overlap with the northernmost parts of that of *C. nitidulum* (Salvini-Plawen, 1975). *Chaetoderma canadense* Nierstrasz (1903), which occurs in the northwestern Atlantic, has also been suggested to be conspecific with *C. nitidulum* (Scheltema, 1972, 1973). Salvini-Plawen (1978) analysed and discussed in detail the species-specific differences in morphology of the North Atlantic *Chaetoderma* species, concluding that they represent valid species and that sclerite morphology is useful for species identification.

Our work on the geographical distribution of caudofoveates in Scandinavian waters (unpublished), however, has brought to our attention that identification of the North Atlantic *Chaetoderma* species is not an easy task and especially that sclerite morphology can vary between specimens of different sizes and from different geographical areas (see also Salvini-Plawen, 1978, for *C. nitidulum* from the Norwegian west coast and from Sweden). In addition,

radula dissections of specimens with *C. nitidulum*-like sclerites have showed varying morphologies. These observations were concordant with observations and comments by other authors (e.g. Ivanov & Scheltema, 2000) and led us to pose a question concerning the status of this species: is *C. nitidulum* a widely distributed species that is variable in morphology, or does it represent a species complex with numerous undescribed species?

To answer this question, we investigated chaetodermatid material from the entire distributional range of *C. nitidulum*, including specimens from close to the type locality on the Swedish west coast. We examined *Chaetoderma* specimens of different sizes and extended the taxon sampling to co-occurring species of *Falcidens*, including a yet-undescribed species. To elucidate the genetic structure within this material, we sequenced three molecular markers for specimens from all sampling localities where material fixed in a manner suitable for genetic work was available. In parallel, we analysed the sclerite and radula morphology of representatives of the resulting clades, supplemented by additional material not suitable for genetic work.

## MATERIAL AND METHODS

In total, 64 specimens of Chaetodermatidae were analysed in the study (Table 1). The material mainly consisted of specimens of *Chaetoderma* from the Eastern Atlantic which, based on morphology, were tentatively identified as *C. nitidulum*. The large morphological variation in the material made species identification based on morphology ambiguous in many cases. In addition, some specimens from the Western Atlantic were included. In overall morphology these specimens were similar to *C. canadense*, which is found in this area, but there were differences between the sclerotome of the juvenile specimens at hand from that described for *C. Canadense*; therefore we refer to them herein as *Chaetoderma* sp. Three species of *Falcidens* were included: *F. crossotus*, *F. caudatus* (a species from the Western Atlantic resembling *F. crossotus* in body shape) and specimens of a putative new *Falcidens* species from Sweden. Sampling sites in the Eastern Atlantic included Gullmarsfjord and Kosterfjord, Sweden; Trondheimsfjord, Porsangerfjord and several fjords near Bergen, Norway; fjords of the Svalbard Archipelago; Moray Firth and Loch Etive, Scotland, and north east of Iceland. Samples from the Western Atlantic were collected from off North Carolina, USA. A map of sampling sites (Fig. 2) was made with Ocean Data View v. 4.7.10 (Schlitzer, 2002).

### *Molecular analyses*

A total of 51 specimens were included in the molecular analyses. From Norwegian and Swedish waters, 37 specimens tentatively identified as *C. nitidulum*, five specimens of *F. crossotus* from Norway and two specimens of the putative new species of *Falcidens* from Sweden were used. In addition, four specimens of *Chaetoderma* sp. and two specimens of *F. caudatus* from the eastern USA were included (Table 1). Tissue samples were taken from the midbody of each specimen, leaving the remaining body parts for morphological analysis. Samples were fixed in 96% ethanol and DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen), according to the manufacturer's instructions. Fragments of the mitochondrial cytochrome *c* oxidase 1 (COI) and 16S SSU rRNA genes were amplified with Takara Ex Taq HS, using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) for COI, and primers 16LRN13398 and 16SRHTB (Koufopanou, Reid & Ridgway, 1999) for 16S. The nuclear ribosomal 18S gene (SSU) was amplified with primers 18e (Hillis & Dixon, 1991) and 18p (Halanych, Lutz & Vrijenhoek, 1998) using Takara LA Taq with a GC rich buffer, to overcome the problem of secondary structures hampering amplification of this gene in aplacophorans when standard protocols are used (see Meyer *et al.*, 2010). The PCR profile for amplification of COI and 16S consisted of an initial step of 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 50°C for 30 s and 72°C for 1 min, and a final elongation step at 72°C for 7 min. For 18S, annealing was at 48°C for 1 min and elongation for 2 min.

To exclude the presence of pseudogenes or gene duplicates, the individual sequence chromatogram files were checked for the presence of double peaks and the COI alignment was translated into amino acids and checked for premature stop codons and frame shifts.

Alignments of each of the gene fragment were made using Muscle (Edgar, 2004). The caudofoveate *Scutopus ventrolineatus* was used as outgroup. Phylogenetic analyses were carried out for each of the sequenced genes separately and with all three genes concatenated. RaxML v. 7.0.3 (Stamatakis, 2006) was used for maximum likelihood (ML) analyses applying the GTRGAMMA model and 100 bootstrap (BS) replicates. Bayesian inference (BI) analyses were carried out in MrBayes v. 3.2 (Huelsenbeck & Ronquist, 2001; Ronquist *et al.*, 2012) with two runs of four chains for 10 million generations, sampling every 1,000 generations. For analyses in MrBayes, the data were partitioned according to gene and appropriate evolutionary models were applied for each unlinked partition (GTR + G for 16S, GTR + G + I for COI and 18S) based on the Akaike Information Criterion (AIC) computed by the program jModeltest (Posada, 2008). To evaluate nodal support, in the ML analysis BS support values ≥75% were considered highly supported, while in the BI analysis posterior probabilities (PP) of ≥0.95 were considered significant.

Haplotype networks were made in PopART (Leigh & Bryant, 2015) using the TCS algorithm (Clement *et al.*, 2002). Pairwise uncorrected p-distances were calculated from COI sequences using MEGA v. 7.0 (Kumar, Stecher & Tamura, 2016).

### *Morphological examination*

Preliminary species identification was made based on sclerite morphology and partly confirmed by radula morphology. Radula and sclerites were prepared as described by Scheltema & Ivanov (2004) and Schander *et al.* (2006). The radula was dissected out and the tissue dissolved in sodium hypochlorite (bleach) and washed in distilled water before mounting in glycerine on microscope slides. Sclerites were removed either from entire specimens by carefully scratching them off with a needle, or by cutting off a piece of the cuticle and letting the tissue dissolve in bleach before washing in distilled water and shaking the sclerites off on a slide, where they were left to dry before mounting. Sclerites and radulae were photographed using a Leica DM6000B microscope with a Leica DFC420 camera; sclerites were photographed under cross-polarized light. Under cross-polarized light, due to birefringence, the sclerites produce coloured bands or isochromes, showing the pattern and thickness of the sclerites (Scheltema & Ivanov, 2004). The colours indicate the following approximate thickness: 1st order: grey to creamy white, 1–2 μm; yellow to yellowish brown, 2–3 μm; magenta, 3.5 μm; 2nd order: blue, 4 μm; green, 5 μm; yellow, 6 μm; orange, 7 μm; magenta, 7.5 μm; 3rd order: blue, 8 μm; green, 9 μm; yellow, 10 μm. In sclerites thicker than 10 μm, alternating bands of pink and green appear. It is important to notice that thickness interpreted from colour charts is somewhat subjective (but is usually accurate to within 1–1.5 μm) and that it is the pattern made by the isochromes that is important for taxonomic purposes.

A total of 31 radula preparations were made. Sclerites were studied in detail from 39 of the specimens included in the study (Table 1). To investigate developmental changes from juvenile to adult specimens, we selected specimens of varying body length that had successfully been sequenced in the molecular analyses. Sclerites from ten specimens sampled from Bergen, western Norway, and radulae from eight specimens from Bergen and six

**Table 1.** Specimens included in this study. Material was loaned from or deposited in the following museums: University Museum of Bergen (ZMUB), NTNU University Museum (Vitenskapsmuseet; VM) and National Museums Scotland (NMSZ).

| | Specimen no. | Locality | Position | Museum no. | Radula | Sclerites | Length (mm) | GenBank accession no. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | COI | 16S rRNA | 18S rRNA |
| *Chaetoderma nitidulum* s.l. | 1 | Kosterfjord, Sweden | 58°35.207′N 11°04.274′E | ZMBN 117086 | – | + | 10.6 | MG264102 | - | - |
| *Chaetoderma nitidulum* s.l. | 2 | Kosterfjord, Sweden | 58°52.424′N 11°06.178′E | ZMBN 117087 | – | + | 14.2 | MG264082 | - | - |
| *Chaetoderma nitidulum* s.l. | 3 | Kosterfjord, Sweden | 58°52.424′N 11°06.178′E | ZMBN 117088 | – | + | 9 | MG264103 | - | MG264026 |
| *Chaetoderma nitidulum* s.l. | 4 | Kosterfjord, Sweden | 58°52.463′N 11°5.009′E | ZMBN 117089 | – | – | n/a | MG264081 | - | - |
| *Chaetoderma nitidulum* s.l. | 5 | Kosterfjord, Sweden | 58°52.463′N 11°5.009′E | ZMBN 117090 | – | – | n/a | MG264108 | - | - |
| *Chaetoderma nitidulum* s.l. | 6 | Kosterfjord, Sweden | 58°52.463′N 11°5.009′E | ZMBN 117091 | – | – | n/a | MG264106 | - | - |
| *Chaetoderma nitidulum* s.l. | 7 | Kosterfjord, Sweden | 58°52.3′N 11°06.67′E | ZMBN 117092 | – | – | n/a | MG264107 | - | - |
| *Chaetoderma nitidulum* s.l. | 8 | Kosterfjord, Sweden | 58°52.3′N 11°06.67′E | - | – | – | n/a | MG264087 | - | - |
| *Chaetoderma nitidulum* s.l. | 9 | Kosterfjord, Sweden | 58°53.032′N 11°6.021′E | ZMBN 117094 | – | – | n/a | MG264114 | - | - |
| *Chaetoderma nitidulum* s.l. | 10 | Kosterfjord, Sweden | 58°87′N 11°09′E | ZMBN 117095 | – | – | n/a | MG264086 | - | - |
| *Chaetoderma nitidulum* s.l. | 11 | Kosterfjord, Sweden | 58°53.032′N 11°6.021′E | - | – | – | n/a | MG264115 | - | - |
| *Chaetoderma nitidulum* s.l.* | 12 | Kosterfjord, Sweden | 58°52.35′N 11°06.67′E | ZMBN 117097 | + | – | n/a | MG264122 | - | - |
| *Chaetoderma nitidulum* s.l. | 13 | Kosterfjord, Sweden | 58°51.73′N 11°05.32′E | ZMBN 117098 | + | + | n/a | MG264080 | - | - |
| *Chaetoderma nitidulum* s.l.* | 14 | Kosterfjord, Sweden | 58°35.207′N11°04.247′E | ZMBN 117099 | + | – | 5.5 | MG264119 | MG264019 | MG264029 |
| *Chaetoderma nitidulum* s.l. | 15 | Gullmarsfjord, Sweden | 58°15.20′N 11°27.20′E | ZMBN 117100 | – | – | n/a | MG264109 | - | - |
| *Chaetoderma nitidulum* s.l. | 16 | Gullmarsfjord, Sweden | 58°15.20′N 11°27.20′E | ZMBN 117101 | – | – | n/a | MG264104 | - | - |
| *Chaetoderma nitidulum* s.l. | 17 | Gullmarsfjord, Sweden | 58°15.20′N 11°27.20′E | ZMBN 117102 | – | – | n/a | MG264101 | - | - |
| *Chaetoderma nitidulum* s.l. | 18 | Gullmarsfjord, Sweden | 58°17.1667′N11°30.967′E | ZMBN 117103 | – | – | n/a | MG264118 | - | - |
| *Chaetoderma nitidulum* s.l. | 19 | Gullmarsfjord, Sweden | 58°17.1667′N11°30.967′E | ZMBN 117104 | – | – | n/a | MG264121 | - | - |
| *Chaetoderma nitidulum* s.l. | 20 | Gullmarsfjord, Sweden | 58°17.1667′N11°30.967′E | ZMBN 117105 | + | + | n/a | MG264112 | - | - |
| *Chaetoderma nitidulum* s.l. | 21 | Bergen, Norway | 60°26.070′N 05°07.441′N | – | – | – | n/a | MG264085 | MG264008 | MG264024 |
| *Chaetoderma nitidulum* s.l. | 22 | Bergen, Norway | 60°19.559′N 05°12.539′E | ZMBN 117106 | + | + | 13.3 | MG264084 | MG264009 | MG264025 |
| *Chaetoderma nitidulum* s.l. | 23 | Bergen, Norway | 60°15.420′N 05°13.145′E | ZMBN 117107 | + | + | 30 | MG264116 | MG264010 | MG264023 |
| *Chaetoderma nitidulum* s.l. | 24 | Bergen, Norway | 60°15.420′N 05°13.145′E | ZMBN 117108 | + | + | 45 | MG264113 | MG264011 | MG264021 |
| *Chaetoderma nitidulum* s.l. | 25 | Bergen, Norway | 60°15.66′N 5°13.2′E | ZMBN 117109 | + | + | 3.1 | MG264105 | - | - |
| *Chaetoderma nitidulum* s.l. | 26 | Bergen, Norway | 60°18.081′N 5°10.400′E | ZMBN 117110 | – | + | 15.5 | MG264083 | - | - |
| *Chaetoderma nitidulum* s.l. | 27 | Bergen, Norway | 60° 16.92′N 5° 12.45′E | ZMBN 117111 | – | + | 16.3 | - | - | - |
| *Chaetoderma nitidulum* s.l. | 28 | Bergen, Norway | 60° 16.13′N 5° 8.88′E | ZMBN 93310 | + | – | n/a | - | - | - |
| *Chaetoderma nitidulum* s.l. | 29 | Bergen, Norway | 60° 24.14′N 4° 56.4′E | ZMBN 93250 | + | + | n/a | - | - | - |
| *Chaetoderma nitidulum* s.l. | 30 | Bergen, Norway | 60° 19.87′N 4° 56.43′E | ZMBN 93251 | + | + | n/a | - | - | - |
| *Chaetoderma nitidulum* s.l. | 31 | Bergen, Norway | 60° 19.87′N 4° 56.43′E | ZMBN 93251 | + | + | n/a | - | - | - |
| *Chaetoderma nitidulum* s.l. | 32 | Sogn og Fjordane, Norway | 60° 52.88′N 4°49.57′E | ZMBN 93255 | + | + | n/a | - | - | - |
| *Chaetoderma nitidulum* s.l. | 33 | Trondheimsfjord, Norway | 63°59.407′N 10°01.348′E | VM 61392 | – | + | 27 | MG264110 | - | - |
| *Chaetoderma nitidulum* s.l. | 34 | Porsangerfjord, Norway | 70°12.14′N 25°26.70′E | ZMBN 117135 | + | + | 7.1 | - | - | - |
| *Chaetoderma nitidulum* s.l. | 35 | Porsangerfjord, Norway | 70°12.14′N 25°26.70′E | ZMBN 117137 | + | + | 10.1 | - | - | - |
| *Chaetoderma nitidulum* s.l. | 36 | Porsangerfjord, Norway | 70°12.14′N 25°26.70′E | ZMBN 117130 | + | + | 20 | - | - | - |
| *Chaetoderma nitidulum* s.l. | 37 | Porsangerfjord, Norway | 79°12.133′N 25°16.016′E | ZMBN 117112 | + | + | 6.2 | MG264100 | - | - |
| *Chaetoderma nitidulum* s.l. | 38 | Porsangerfjord, Norway | 79°12.133′N 25°16.016′E | ZMBN 117113 | + | + | 9.5 | MG264117 | - | - |

*Continued*

**Table 1.** *Continued*

| | Specimen no. | Locality | Position | Museum no. | Radula | Sclerites | Length (mm) | GenBank accession no. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | COI | 16S rRNA | 18S rRNA |
| *Chaetoderma nitidulum* s.l. | 39 | Porsangerfjord, Norway | 79°12.133′N 25°16.016’E | ZMBN 117114 | + | + | 17.5 | MG264097 | - | - |
| *Chaetoderma nitidulum* s.l. | 40 | Svalbard, Norway | 79°38.183′N 19°45.899’E | ZMBN 117115 | – | – | n/a | MG264094 | - | - |
| *Chaetoderma nitidulum* s.l. | 41 | Svalbard, Norway | 79°38.183′N 19°45.899’E | ZMBN 117116 | + | + | 13 | MG264092 | MG264013 | MG264030 |
| *Chaetoderma nitidulum* s.l. | 42 | Svalbard, Norway | 79°35.371′N 18°51.983’E | ZMBN 117117 | – | + | n/a | MG264120 | MG264012 | MG264031 |
| *Chaetoderma nitidulum* s.l. | 43 | Svalbard, Norway | 79°41.619′N 11°06.873’E | ZMBN 117118 | + | + | n/a | MG264093 | MG264014 | |
| *Chaetoderma nitidulum* s.l. | 44 | Svalbard, Norway | 81°00.067′N 19°17.799’E | ZMBN 117119 | + | + | 10.5 | MG264095 | MG264015 | MG264028 |
| *Chaetoderma nitidulum* s.l. | 45 | Svalbard, Norway | 80°06.513′N 22°08.481’E | ZMBN 117120 | + | + | 9.5 | MG264098 | MG264016 | MG264027 |
| *Chaetoderma nitidulum* s.l. | 46 | Svalbard, Norway | 81°00.067′N 19°17.799’E | ZMBN 117121 | + | + | 18 | MG264096 | MG264018 | |
| *Chaetoderma nitidulum* s.l. | 47 | Svalbard, Norway | 80°09.141′N 16°56.126’E | ZMBN 117122 | + | + | 8.8 | MG264111 | MG264017 | MG264022 |
| *Chaetoderma nitidulum* s.l. | 48 | North East Iceland | 66°18.06′N 12°22.40’W | ZMBN 117123 | – | + | 12.4 | MG264099 | - | - |
| *Chaetoderma nitidulum* s.l. | 49 | Loch Etive, Scotland | 56°29,88′N 5°8.80’W | NMSZ-1999-0071.137 | + | + | n/a | - | - | - |
| *Chaetoderma nitidulum* s.l. | 50 | Moray Firth, Scotland | 57°32.34′N 4°8.57’W | NMSZ-1984-053.012/1 | + | + | n/a | - | - | - |
| *Chaetoderma nitidulum* s.l. | 51 | Moray Firth, Scotland | 57°32.34′N 4°8.57’W | NMSZ-1984-053.012/2 | + | + | n/a | - | - | - |
| *Chaetoderma nitidulum* s.l. | 52 | Edinburgh, Scotland | 56°06.0′N 02°07.3’W | NMSZ-2000279.445/1 | + | + | n/a | - | - | - |
| *Chaetoderma nitidulum* s.l. | 53 | Edinburgh, Scotland | 56°06.0′N 02°07.3’W | NMSZ-2000279.445/2 | + | + | n/a | - | - | - |
| *Chaetoderma nitidulum* s.l. | 54 | North Carolina, USA | 36°28.466′N 74°46.746’W | ZMBN 117124 | – | + | 3.5 | MG264091 | - | - |
| *Chaetoderma nitidulum* s.l. | 55 | Southern New England, USA | 39°54.085′N 69°54.601’W | ZMBN 117125 | – | + | 5.2 | MG264089 | - | - |
| *Chaetoderma nitidulum* s.l. | 56 | Southern New England, USA | 39°54.085′N 69°54.601’W | - | – | + | n/a | MG264090 | - | - |
| *Chaetoderma nitidulum* s.l. | 57 | Southern New England, USA | 39°54.085′N 69°54.601’W | ZMBN 117127 | – | + | n/a | MG264088 | MG264020 | MG264034 |
| *Falcidens caudatus* | 1 | North Carolina, USA | 35°28.466′N 074°46.746’W | ZMBN 117128 | | | | MG264124 | MG264005 | MG264035 |
| *Falcidens caudatus* | 2 | North Carolina, USA | 35°28.466′N 074°46.746’W | ZMBN 117129 | | | | MG264123 | - | - |
| *Falcidens crossotus* | 1 | Bergen, Norway | 60°26.070′N 05°07.441’E | ZMBN 117131 | | | | MG264126 | - | - |
| *Falcidens crossotus* | 2 | Bergen, Norway | 60°26.070′N 05°07.441’E | ZMBN 117132 | | | | MG264128 | - | - |
| *Falcidens crossotus* | 3 | Bergen, Norway | 60°26.070′N 05°07.441’E | ZMBN 117133 | | | | MG264129 | MG264006 | MG264037 |
| *Falcidens crossotus* | 4 | Sogn og Fjordane, Norway | 60°57.986′N 4°40.912’E | ZMBN 117134 | | | | MG264125 | - | - |
| *Falcidens crossotus* | 5 | Nordland, Norway | 67°50.810′N 11°48.850’E | ZMBN 93386 | | | | MG264127 | MG264007 | MG264036 |
| *Scutopus ventrolineatus* | - | Bergen, Norway | 60°21.8298′N 4°54.7992’E | ZMBN 117136 | | | | MG264130 | MG264004 | MG264038 |

Specimens originally identified as *Falcidens* are marked with an asterisk.

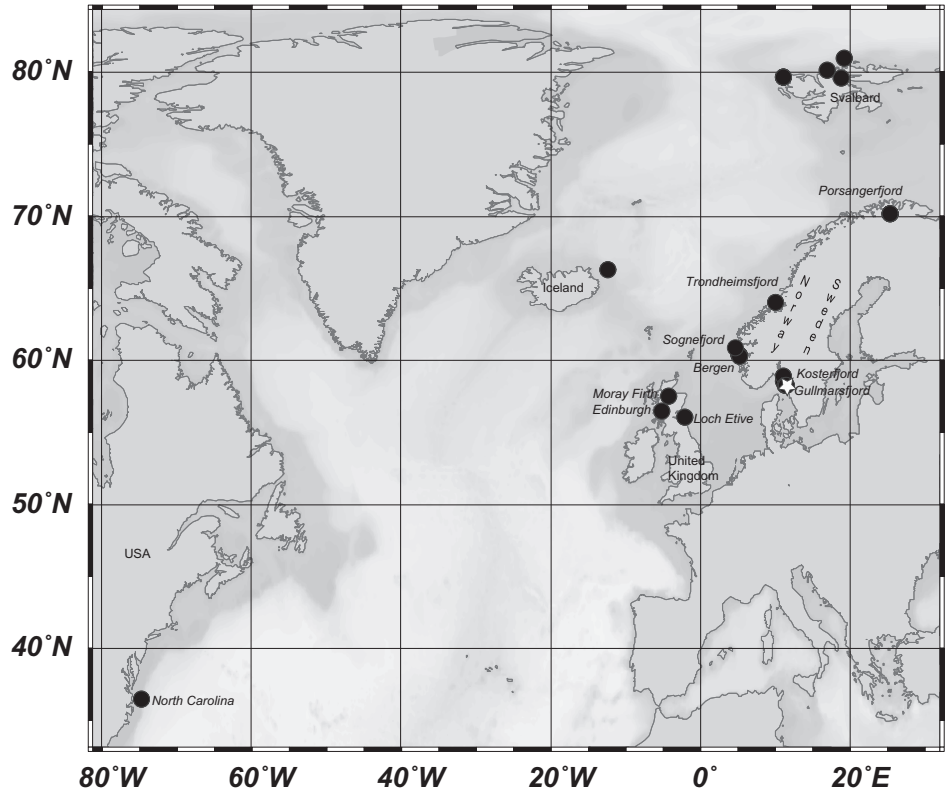


**Figure 2.** Map of *Chaetoderma nitidulum s. l.* sampling sites. Black filled circles indicate sampling localities. White star indicates type locality of *Chaetoderma nitidulum* (Lovén, 1844).

specimens from Porsangerfjord, northern Norway, were compared in detail.

For histological sectioning, specimens fixed in 4–8% formalin and preserved in 70% ethanol were decalcified overnight in a dilute solution of hydrochloric acid in 70% ethanol (1 drop of 37.2% HCl per 5 ml of 70% ethanol) and stained with rose bengal (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein), followed by a stepwise dehydration in ethanol and embedding in Agar low-viscosity resin (Agar Scientific). After polymerizing for around 16 h at 70 °C, the resin blocks were trimmed and transverse section series (2 µm thickness) were made for the anterior body region containing the radula, using a Leica 2255 rotation microtome with a Diatome Histo Jumbo diamond knife. Sections were stained with toluidine blue. Histological sections were photographed on a Leica DM 6000B microscope with a Leica DFC 420 digital camera using differential interference contrast (DIC) or brightfield light settings.

## RESULTS

### *Molecular analyses*

Analysis of COI (Fig. 3) and concatenated analysis of COI, 16S and 18S markers (Fig. 4) resulted in phylogenetic trees with the same topology. The single-gene analyses of 16S (not shown) and 18S (Fig. 5) resulted in trees with lower resolution. ML and BI analyses resulted in the same tree topologies. *Falcidens crossotus* and *F. caudatus* are resolved as sister species in the trees and are separated by a large genetic distance from a clade comprising all *Chaetoderma* specimens, which we refer to as *C. nitidulum s. l.* This clade is separated into two main subclades. The first (clade I) is composed of all Eastern Atlantic *Chaetoderma* specimens and, surprisingly, the specimens previously classified as an undescribed species of *Falcidens* from Sweden. The second clade (clade II) comprises the specimens of *Chaetoderma* sp. from the Western Atlantic. The Eastern Atlantic clade comprises two well-defined subclades, which we refer to as *C. nitidulum* Ia and Ib. The three clades of *Chaetoderma* are similarly revealed in the two haplotype networks, based on COI and 16S (Fig. 6). The *C. nitidulum* Ia clade includes specimens from all Eastern Atlantic localities (southern Sweden and the entire Norwegian coastline to the Svalbard Archipelago), while the *C. nitidulum* Ib clade comprises specimens from only the southernmost localities, from Sweden and as far north as Bergen (Fig. 4, Table 1). The division into two *C. nitidulum* clades is highly supported by BS and PP values. In the more conserved 18S gene, the genetic variation is minimal. Analysed separately, the 18S gene (Fig. 5) provides support for the monophyly of *Falcidens* and *Chaetoderma*, but resolution within *Chaetoderma* is poor. The *C. nitidulum* I and II clades are not recovered, as the single specimen of *C. nitidulum* II clusters within the *C. nitidulum* Ia clade. *Chaetoderma nitidulum* Ia is separated into two subclades, one of them sister to a monophyletic *C. nitidulum* Ib clade.

Genetic distance in the COI gene within each of the two Eastern Atlantic *C. nitidulum* subclades (*C. nitidulum* Ia and Ib) is below 1.2%, while the distance between specimens from different subclades is 5.8–7%. The distance between the Western Atlantic clade II and either of the Eastern Atlantic clades is within the same range (5.6–6.8%). The distance within *F. crossotus* and *F. caudatus* is 0–3.9% and 0.2%, respectively, and the difference between the two *Falcidens* species is 15.6–16.1%.

### *Radula morphology*

Investigation of specimens assigned to *C. nitidulum s. l.* based on molecular analyses and ranging in body length from 3.1 mm to 45 mm showed differences in the radula morphology between specimens of different sizes (Fig. 7). In small, juvenile specimens of up to 10 mm, the radula consists of a short, sclerotized cone (Fig. 8A),

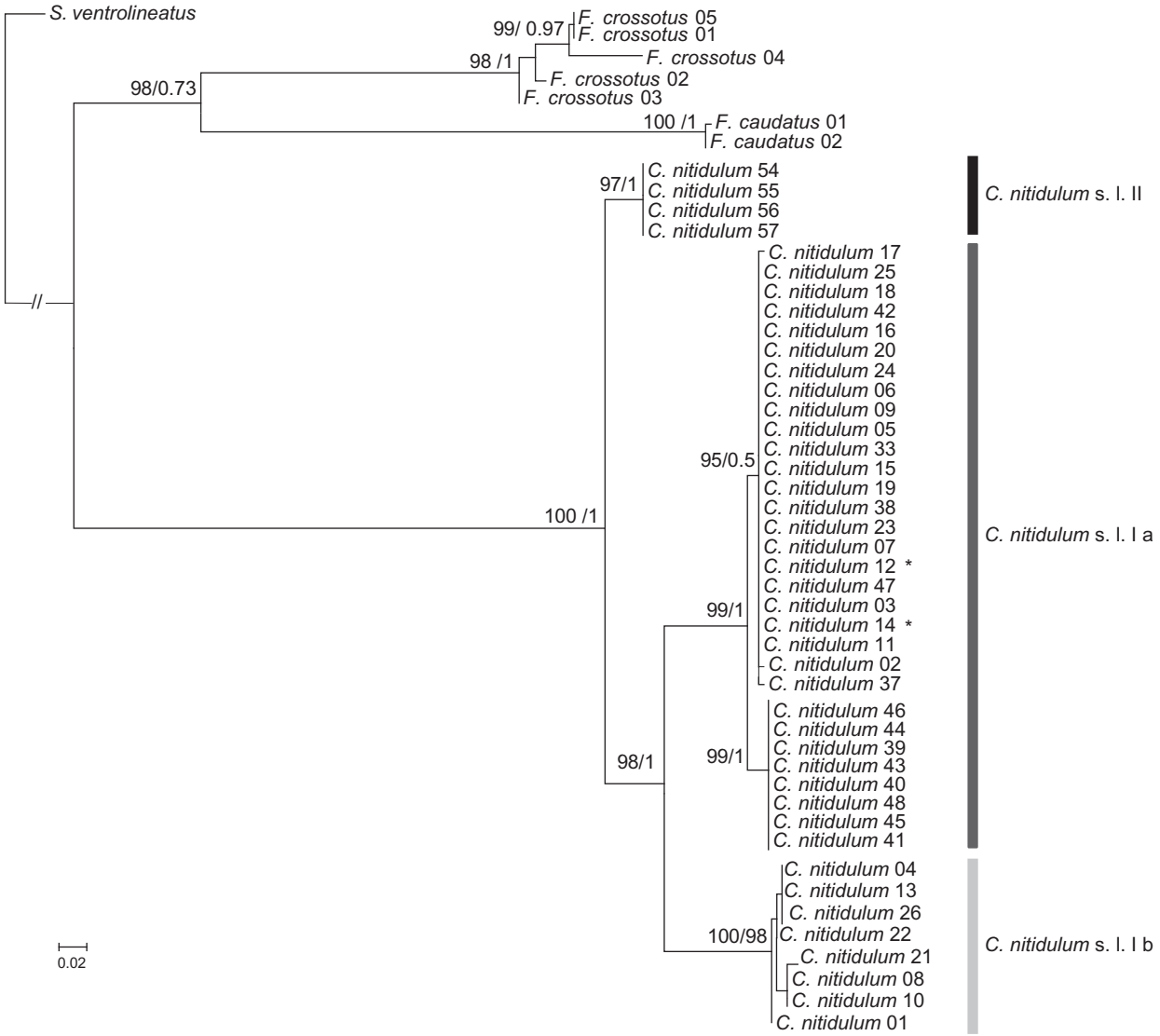


**Figure 3.** Phylogenetic tree inferred from COI sequences. Support values on branches are BS and PP (from ML and BI analyses, respectively). Specimens initially identified as *Falcidens* sp. (Sweden) are marked with an asterisk.

a pair of sclerotized teeth (Fig. 8C–F) and a sclerotized plate located behind the pair of teeth (Fig. 8F). These sclerotized parts are yellow to brown in colour, both in radula preparations (Fig. 7) and histological sections (Figs 8, 9). The connection between the sclerotized parts is composed of cuticular material (Fig. 8B–F), and both teeth and the plate are thus flexible in relation to the cone. The teeth are not connected by a sclerotized symphysis. The epithelium secreting the cuticular sheath is continuous with the pharyngeal epithelium. The cuticular sheath and epithelium together form a dome that is horseshoe-shaped in cross-section over most of its extension (Fig. 8C–E), but has short, paired tips that end just below the level of the tips of the teeth. The dome is filled with musculature (Figs 8B, C, 9A). The cuticle is thickest in its lower, lateral areas (Figs 8B, 9B). In larger specimens, the cuticular material connecting the teeth to the cone is strengthened and elongated to form so-called lateral projections (Scheltema, 1972). The cone and the entire dome appear to grow continuously during ontogeny, while the teeth stay the same size. The paired tips of the dome grow to a level above the tips of the teeth (Fig. 7, specs 22–24). The sclerotized plate is only present in small specimens (up to 12 mm). It is not known if the plate is subsequently dissolved or shed. The larger the animal and its radula grow, the larger the distance between the cone and the teeth becomes and the more voluminous the dome (Fig. 9C–E). In specimens of more than 40 mm, the lateral projections are less obvious and reduced to thin strings, so that the teeth appear to be attached directly to the surface of the paired tips of the cuticular dome (Fig. 7, spec. 24). At the same time, the thickened lateral areas of the dome become increasingly darker in colour, which points to sclerotizing processes. In medium-sized and large specimens the teeth are attached just below the paired tips of the dome (Figs 7, 9F, specs 23, 24). The teeth of large specimens are often slightly shorter than in small specimens and sometimes the tips look broken or worn. In two cases out of 31 specimens, only one tooth was present.

### *Sclerite morphology*

The material of *C. nitidulum s. l.* from Bergen showed differences in sclerite morphology between specimens of different sizes (Figs 10, 11). In small juveniles (1–2 mm; Fig. 10A), the fish-shaped sclerites typical for the neck region of *C. nitidulum* (see below; 'bomb-like

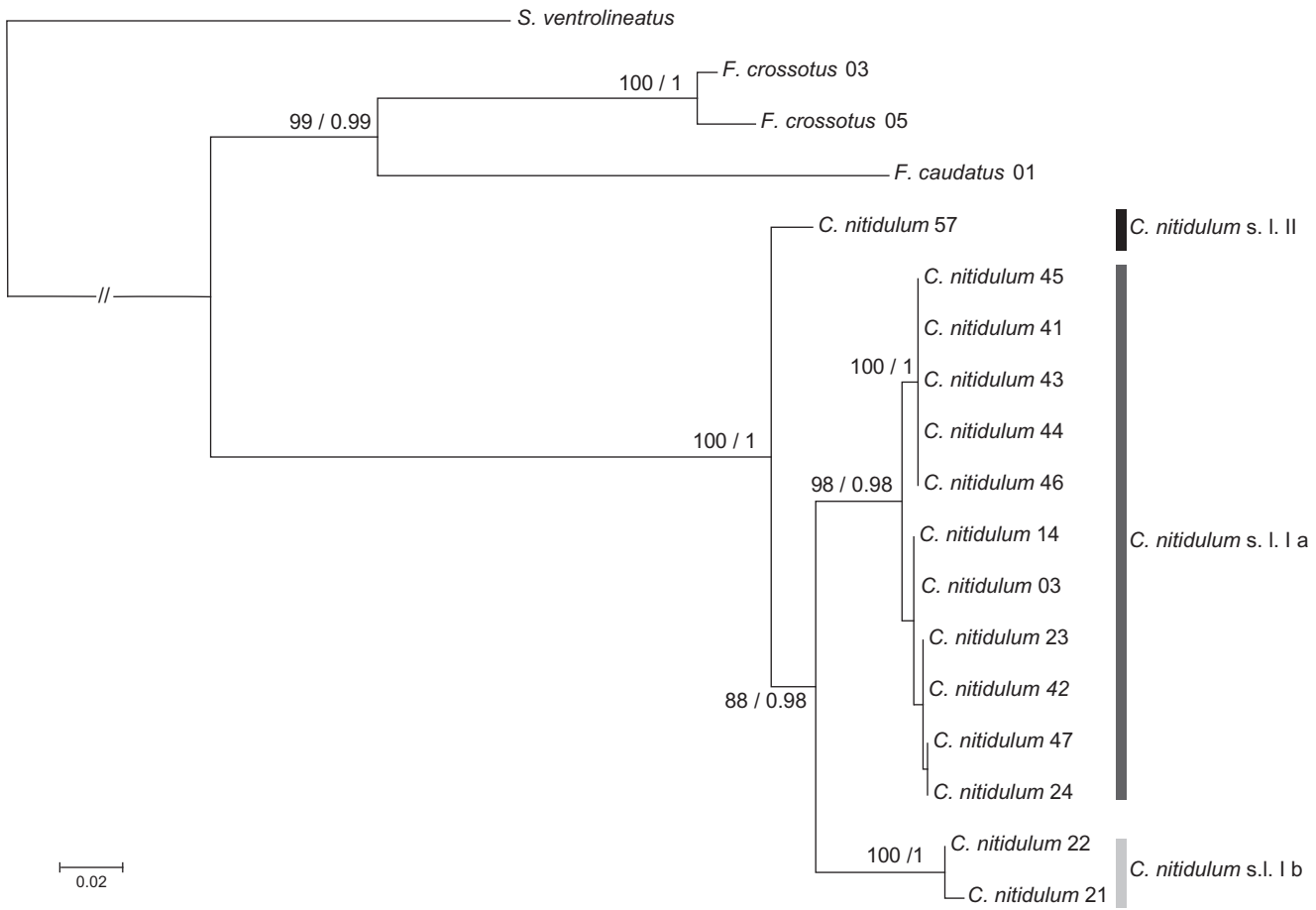


**Figure 4.** Phylogenetic tree inferred from sequences from concatenated dataset of COI, 16S and 18S. Support values on branches are BS and PP (from ML and BI analyses, respectively).

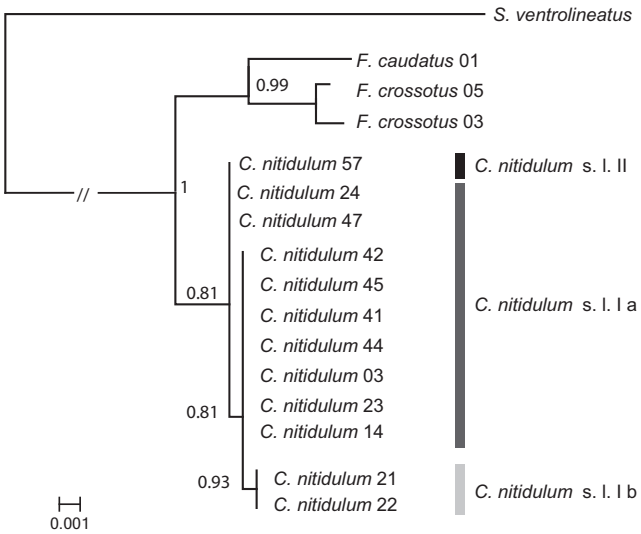


**Figure 5.** Phylogenetic tree inferred from 18S sequences. Support values on branches are BS and PP (from ML and BI analyses, respectively).

spicules', Salvini-Plawen, 1978) are not present, but these become increasingly numerous in larger specimens (Figs 10B, 11). Similarly, in the smallest specimens the long, rod-shaped posteriormost sclerites surrounding the mantle cavity were not found (Fig. 10A), while these are present in increasing numbers in larger specimens (Figs 10B, 11). In small specimens, the sclerites are longer relative to body size, giving juvenile specimens a 'furry' appearance, which gradually changes into a smoother appearance as the animal grows. Despite this, investigations of the individual sclerites reveal that these also continue to grow throughout the lifespan of the animals, and increase in thickness and in length, as the animal grows larger. This is especially evident in the sclerites from the posterior trunk and the sclerites surrounding the mantle cavity opening, which become increasingly elongated in larger specimens (compare Fig. 11A, B).

Sclerites of *C. nitidulum s. l.* from different geographical regions show a gradual difference in sclerite morphology with changing latitude (Figs 10–12). In Eastern Atlantic specimens from the southern regions, sclerites in the neck region consist of relatively broad, fish-shaped sclerites with an indented waist and a rounded, broad tip, which are interspersed with broad, triangular scales (Fig. 12A). In specimens from the more northern regions, the sclerites in the neck region consist of asymmetrical, elongated, triangular scales, which are lacking in the southernmost specimens, and very slender fish-shaped scales (Fig. 12B). The sclerites from the trunk region in specimens from the southern localities are broad, mostly with distinct striation and a weak central keel (Fig. 12A). Sclerites from the posterior trunk have broad bases, which are often flat or slightly indented. In specimens from the northern regions, trunk sclerites are slender with a distinct keel, or sometimes with three distinct ridges, while striation, when present, is often weak. These characteristics are most pronounced in the northernmost specimens, from Porsangerfjord (Finnmark, Norway) and the Svalbard Archipelago (Fig. 12B). Differences in sclerite morphology

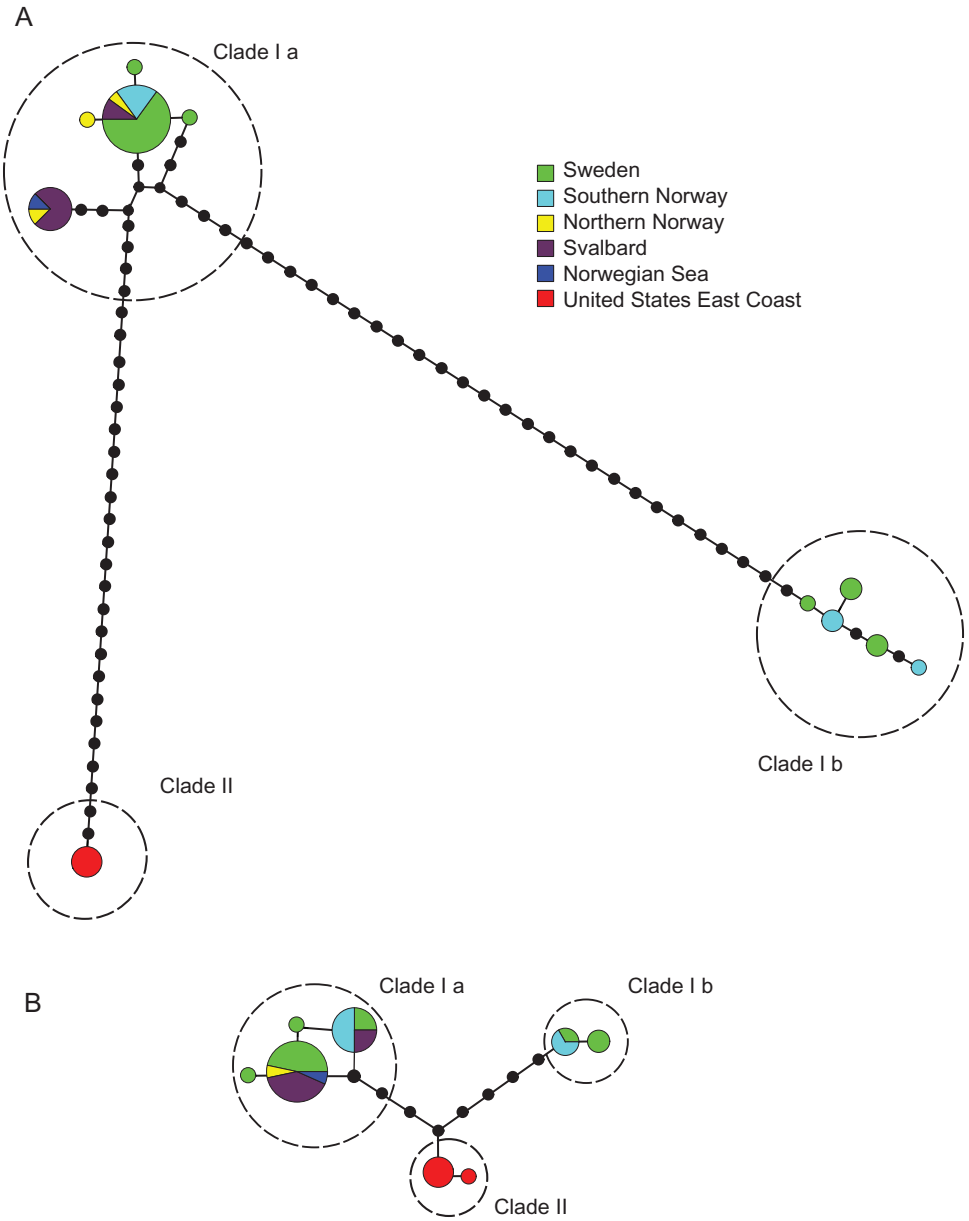


**Figure 6. A.** Haplotype network for COI sequences. **B.** Haplotype network for 16S sequences. Identical haplotypes are grouped together; size of circle represents the number of haplotypes in each group. Black, filled circles represent hypothetical missing haplotypes between groups. Geographical origin of sequences is indicated by colour.

between specimens from different geographical regions in the Eastern Atlantic do not reflect the two molecular clades Ia and Ib.

Sclerites from our specimens from the Western Atlantic (Fig. 13), which were all juvenile, are generally broader and flatter than in specimens from the Eastern Atlantic. The scales from the anterium are broader with more pointed tips than those in the Eastern Atlantic specimens. The sclerites from the neck are broader and more triangular, with a less indented waist. The fish-shaped sclerites typical of *C. nitidulum* are not found in these specimens, as in other juveniles investigated. Sclerites from the anterior trunk are broader, with straight or convex sides, rather than concave sides indented to a waist that are found in the Eastern Atlantic specimens. Sclerites from the posterior trunk are broad, with a central keel as in Eastern Atlantic specimens, but here the sclerites are smoother with almost no striation, and the base is flat and sometimes slightly indented.

## DISCUSSION

Our integrative analysis of molecular and morphological data in North Atlantic chaetodermatids reveals a very distinct genetic structure, which is not always consistent with our preliminary species identification based on sclerite and radula characters.

Most strikingly, specimens that we had considered to represent a new species of *Falcidens* turned out to cluster within the *Chaetoderma nitidulum* Ia subclade. Also, several specimens with a radula that did not correspond to either a *Falcidens* radula or a *Chaetoderma* radula, but rather showing an intermediate morphology without a central plate but with large denticles relative to body size, turned out to be nested within *C. nitidulum s. l.* A comparison of specimens from different size classes among our samples revealed that all specimens of the presumed new species of *Falcidens* were very small, with body lengths under 6 mm, while specimens unambiguously identified as *C. nitidulum* were larger

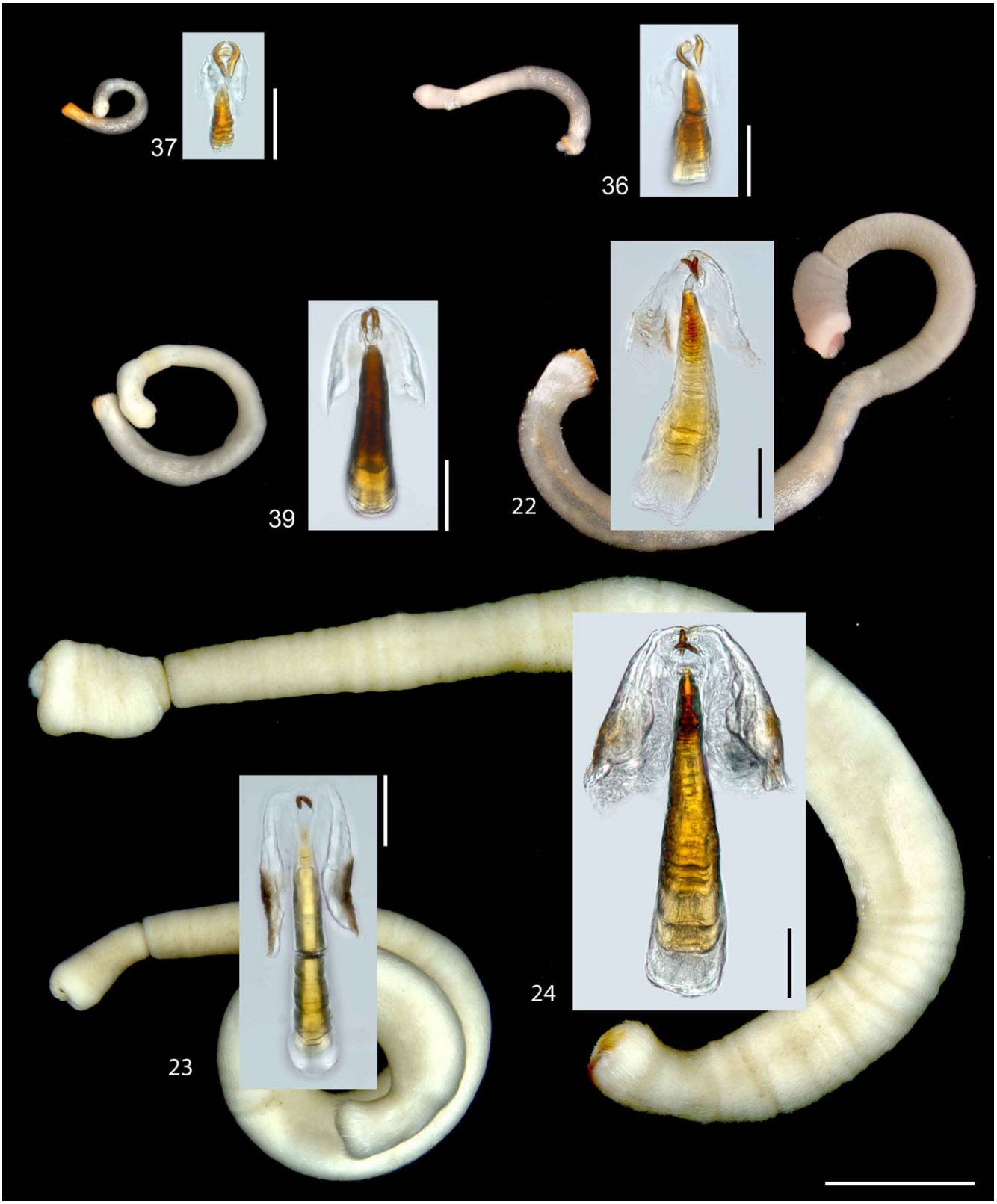


**Figure 7.** Radula morphology of different-sized specimens of *Chaetoderma nitidulum*. Numbers correspond to specimen numbers in Table 1. Scale bar habitus = 5 mm, scale bar radulae = 0.1 mm.

than 15 mm, while the specimens that could not be unambiguously assigned to either *Falcidens* or *Chaetoderma* based on radula morphology were of intermediate size. A detailed comparison of the radula morphology and scleritome of specimens ranging from 3 mm to over 40 mm showed that there is a continuous change from a (juvenile) '*Falcidens*-type' radula morphology, via a (subadult) intermediate stage to the (adult) '*Chaetoderma*-type' morphology.

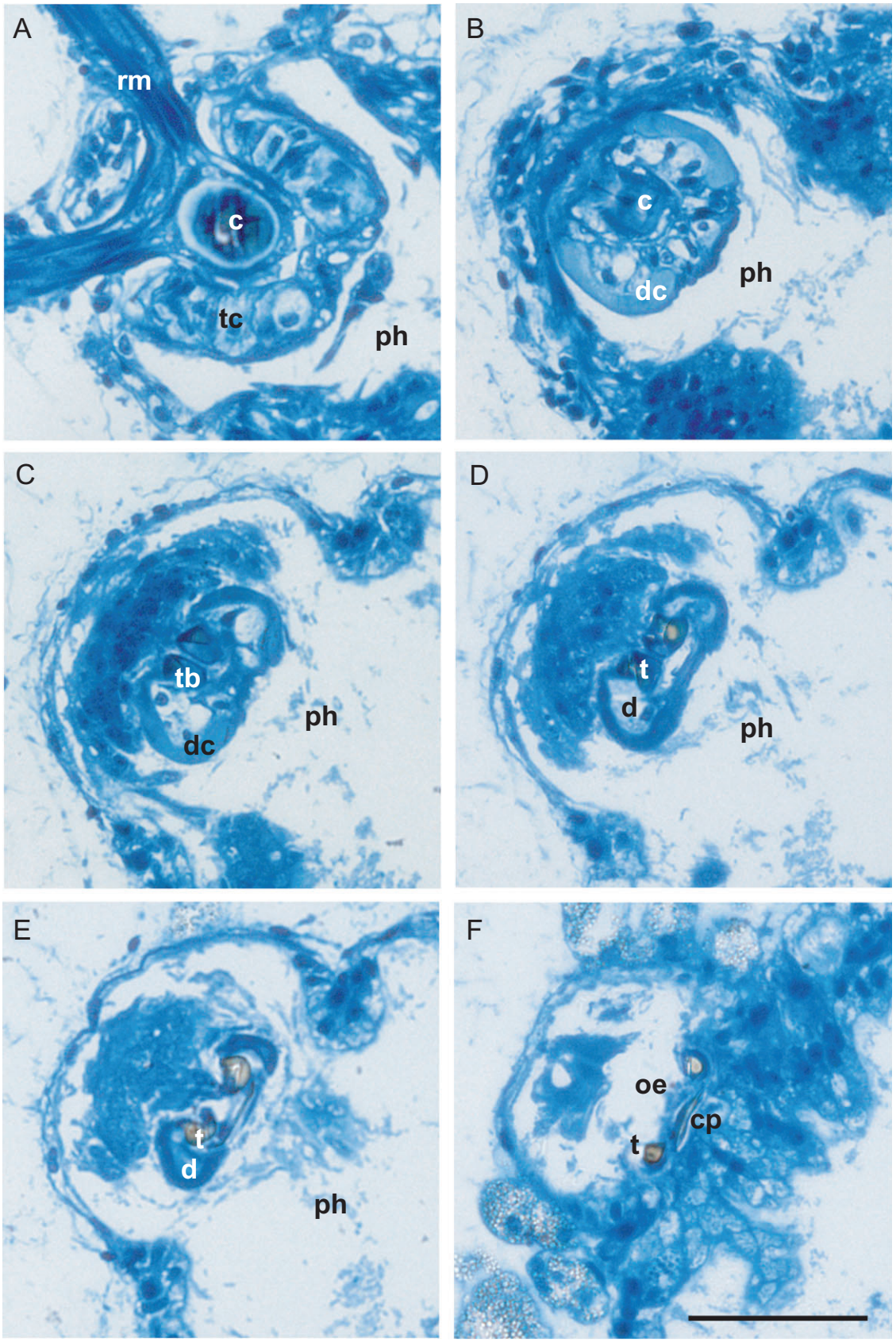


**Figure 8.** Histological cross-sections of radula apparatus of juvenile specimen of *Chaetoderma nitidulum*. **A.** Section through cone (c) and radula musculature (rm). Note turgescent cells (tc) of dome. **B.** Section through tip of cone and dome cuticle (dc) in pharynx (ph). **C.** Section through tooth bases (tb). **D, E.** Sections through teeth (t) and tip of dome (d). **F.** Section through teeth, central plate (cp) and oesophagus (e). Scale bar = 50 µm.

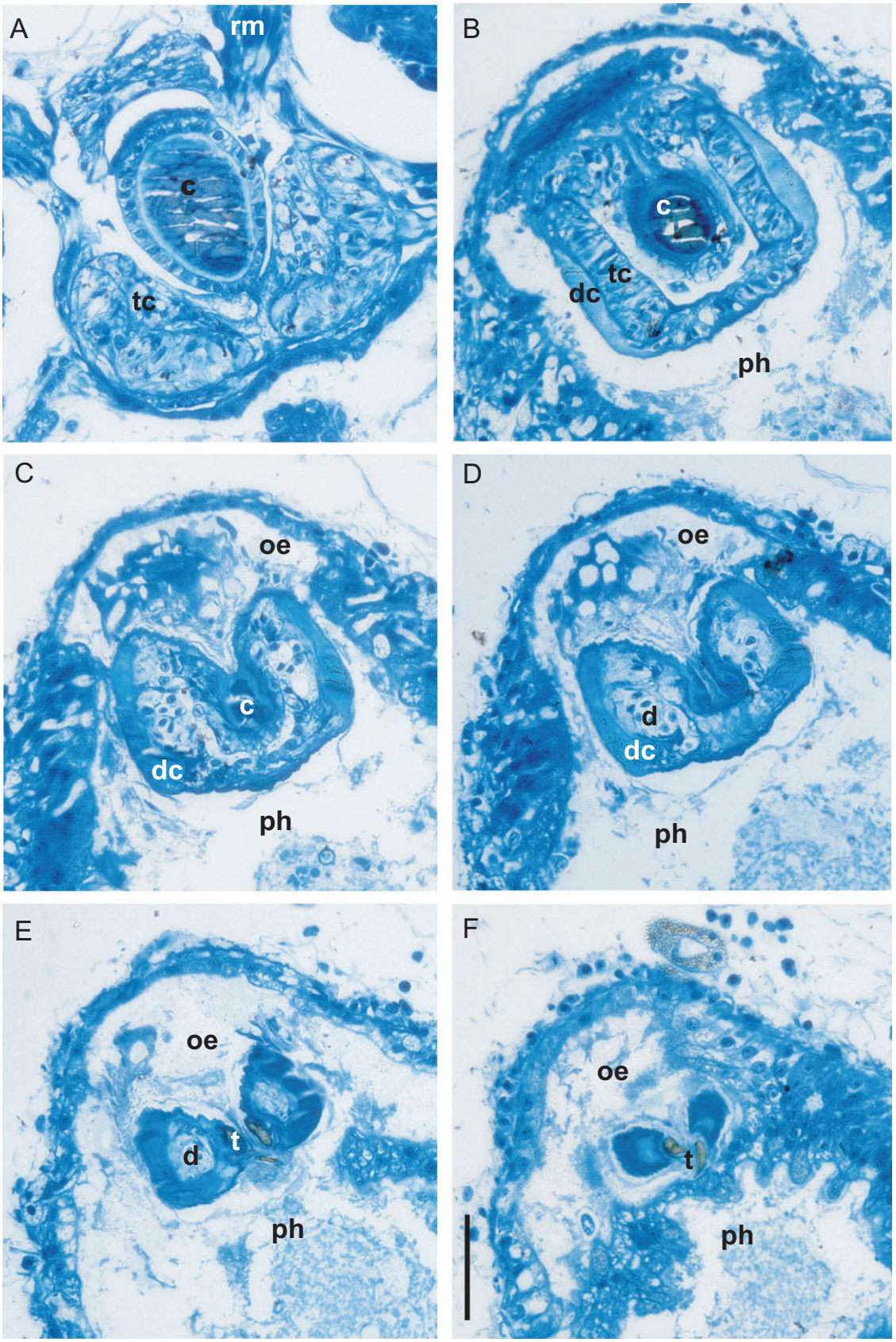


**Figure 9.** Histological cross-sections of radula apparatus of an adult specimen of *Chaetoderma nitidulum*. **A.** Section through cone (c) and radula musculature (rm). Note turgescent cells (tc) of dome. **B.** Section through tip of cone and dome cuticle (dc) in pharynx (ph). **C.** Section through tip of cone, dome cuticle and oesophagus (e). **D.** Section through cone with thickened dome cuticle. **E, F.** Sections through paired tips of dome (d) with teeth (t). Scale bar = 100 μm.

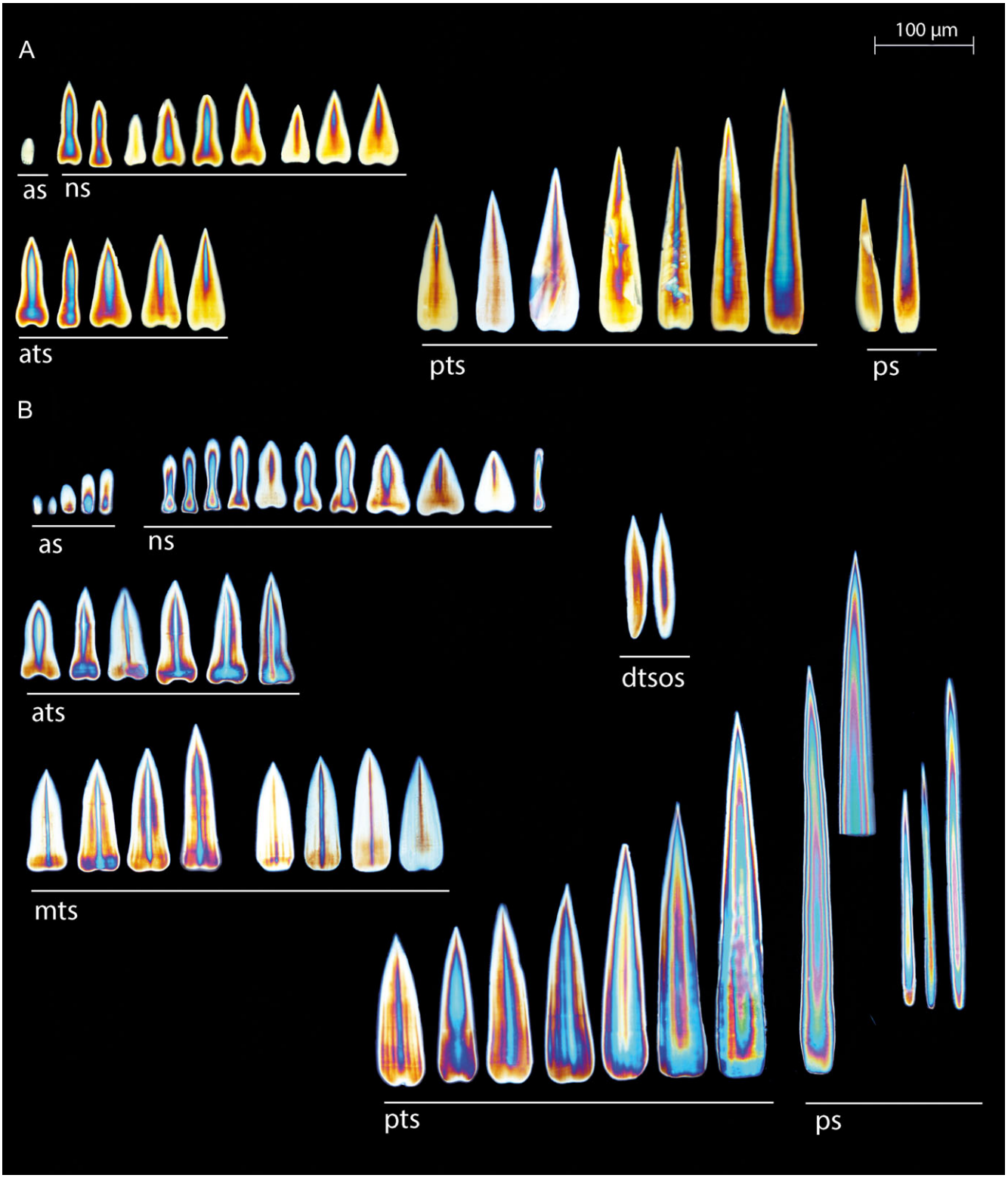


**Figure 10.** Sclerites from specimens of *Chaetoderma nitidulum* from Bergen, Norway. **A.** Specimen 25, body length 3.1 mm. **B.** Specimen 27, body length 16.3 mm. Abbreviations: as, anterium sclerites; ns, neck sclerites; ats, anterior trunk sclerites; mts, mid-trunk sclerites; pts, posterior trunk sclerites; ps, posterium sclerites; dtsos, dorso-terminal sense organ sclerites. Scale bar = 0.1 mm.

*Molecular phylogenetics of North Atlantic Chaetodermatidae*

The genetic variation of COI within each of the clades of *C. nitidulum s. l.* is minimal compared to the genetic distance between the clades. The overall genetic distance within *C. nitidulum s. l.* is considerably higher than within the *Falcidens* species sequenced, but the genetic variability of these species might be underrepresented, as only a few specimens from close localities were sequenced. Representatives of

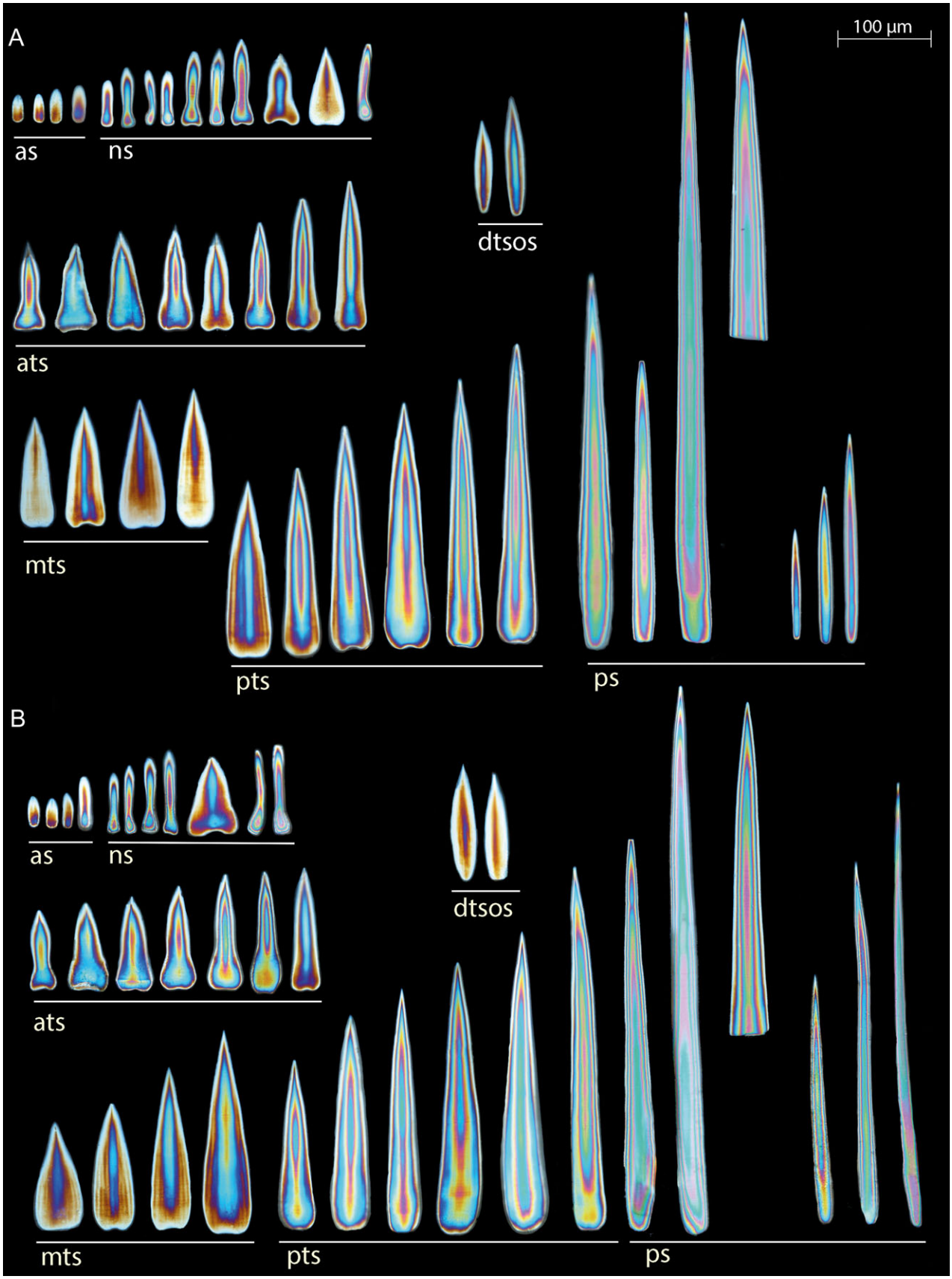


**Figure 11.** Sclerites from specimens of *Chaetoderma nitidulum* from Bergen, Norway. **A.** Specimen 23, body length 30 mm. **B.** Specimen 24, body length 45 mm. Abbreviations as in Figure 10. Scale bar = 0.1 mm.

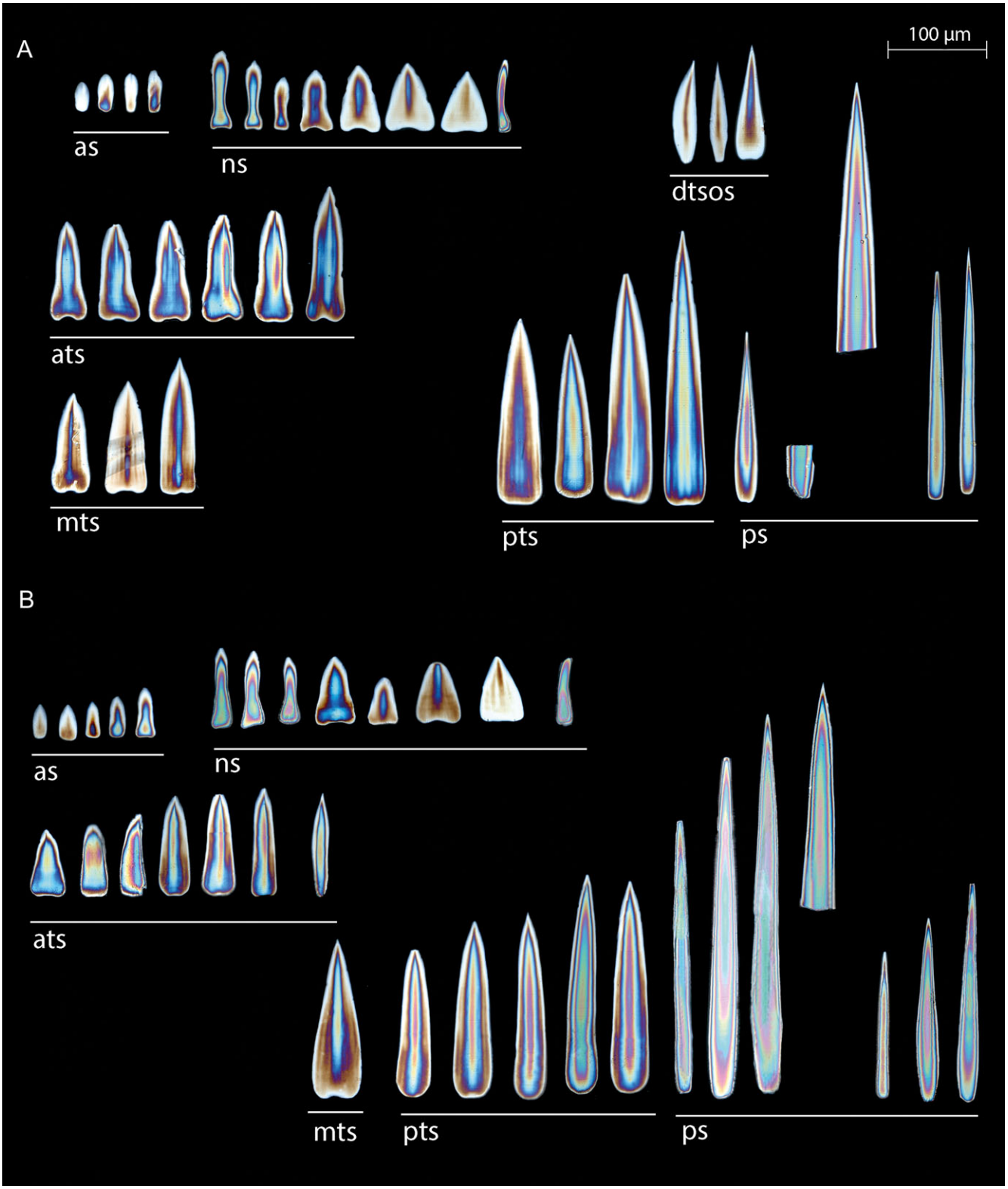


**Figure 12. A.** Sclerites from specimen of *Chaetoderma nitidulum* from Tjärnö, Sweden. Specimen 03, body length 9 mm. **B.** Sclerites from specimen of *C. nitidulum* from Svalbard, Norway. Specimen 41, body length 13 mm. Abbreviations as in Figure 10. Scale bar = 0.1 mm.

the two subclades *C. nitidulum* Ia and Ib in Scandinavia occur sympatrically and specimens from one sampling site may show genetic distances comparable with the distance to specimens found on the opposite side of the Atlantic. Despite these comparable genetic distances in COI, subclades Ia and Ib are supported as sisters, so are more closely related to each other than to the Western Atlantic clade II. The analyses of 18S, however, do not resolve the relationships between the clades of *C. nitidulum s. l.* and this gene is probably too conserved to have diverged between the clades.

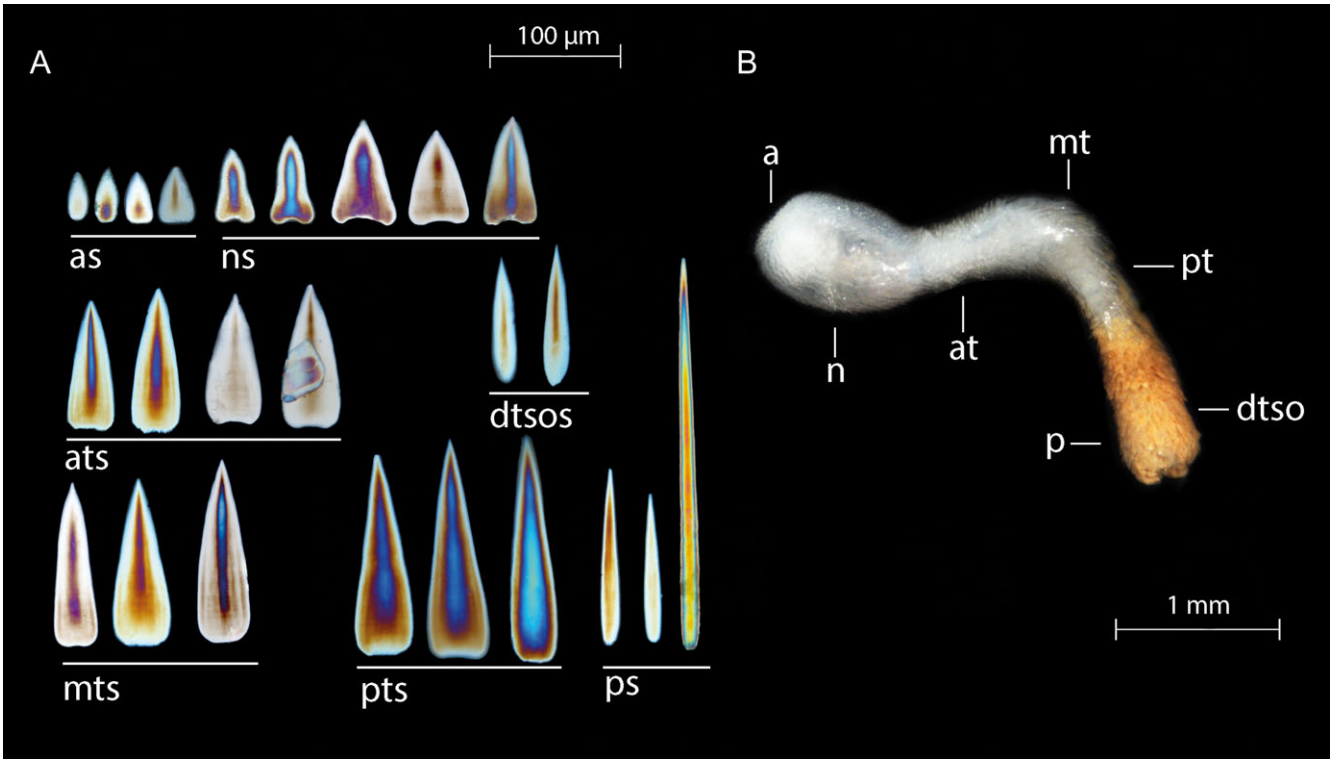


**Figure 13.** Sclerites and habitus of specimen of *Chaetoderma* sp. from North Carolina, USA. Specimen 54, body length 3.5 mm. **A.** Sclerites. **B.** Habitus. Abbreviations: a, anterium; n, neck; at, anterior trunk; pt, posterior trunk; p, posterium; dtso, dorso-terminal sense organ; as, anterium sclerites; ns, neck sclerites; ats, anterior trunk sclerites; mts, mid-trunk sclerites; pts, posterior trunk sclerites; ps, posterium sclerites; dtsos, dorso-terminal sense organ sclerites. Scale bars: **A** = 0.1 mm; **B** = 1 mm.

The genetic distance in COI between *C. nitidulum* clades I and II is considerably lower than the distance between *F. crossotus* and *F. caudatus*, which also inhabit opposite sides of the Atlantic and share many morphological similarities. Even though *F. crossotus* has a more limited distribution, the intraspecific distance within *F. crossotus* is higher than the distances within the clades of *C. nitidulum s. l.*, but in the data from the limited samples available from *F. crossotus* there is no indication of distinct genetic groups.

Up until now, nothing has been known about genetic distances within and between aplacophoran mollusc species. Without this baseline, it is difficult to evaluate the distances found within our samples, but the distance between the *Falcidens* species is significantly higher than the distance between the *C. nitidulum* clades. In other molluscs, estimates of cutoff values in COI distance for differentiating between intra- and interspecific distances are varied. Hebert, Ratnasingham & Waard (2003) first suggested the COI distance between congeneric species of molluscs to be 11.1 ± 5.1%. It has been proposed that threshold values can be used to delimit species (Hebert *et al.*, 2003, 2004), but this approach has since been criticized due to the large variation and often extensive overlap of intra- and interspecific distances (e.g. Meyer & Paulay, 2005; Hickerson, Meyer & Moritz, 2006).

Levels of genetic distance have, however, been assessed in several studies as a part of integrative approaches that combine molecular data with morphological and ecological characters. In gastropods, distances between 6–8% (Krug *et al.*, 2013) and 10% (Malaquias & Reid, 2008, 2009) have been shown to be applicable as cutoff values to separate species, while Puillandre *et al.* (2009) found a 'barcode gap' between intraspecific- and interspecific distance in the range 2.5–7.5%. However, wider ranges of intraspecific distance have also been reported in gastropods, e.g. Jörger *et al.* (2012) estimated a range of intraspecific distances of 1.8–8.7%. In contrast, the reported distances in cephalopods are low, with reported intraspecific distances rarely higher than 1%, and interspecific distances generally ranging between 2% and 3.5% (Strugnell, Collins & Allcock, 2008; Allcock *et al.*, 2011; Amor *et al.*, 2014) or occasionally higher (6.7–7.7%, Undheim *et al.*, 2010). In Polyplacophora, reported distances are considerably higher, distances between congeneric species ranging from 9–17% (Bonfitto *et al.*, 2011) to 11–18% (García-Ríos *et al.*, 2014), while intraspecific distances of up to 8% have been reported (García-Ríos *et al.*, 2014).

Compared to the genetic distances reported in other mollusc groups, the distances in COI between the clades of *C. nitidulum s. l.* do not necessarily indicate a division into separate species, but the distances are high considering that specimens from both of the Scandinavian clades occur sympatrically in part of the distributional range. The gap between the distances within and between subclades Ia and Ib clearly shows that they represent genetically divergent lineages. *Chaetoderma nitidulum s. l.* in Scandinavia consists of two genetically separated populations, one limited to the southern part of the range and the other extending all the way to the northern limit of the distribution. This separation into two subclades cannot readily be attributed to sediment type, depth or other abiotic factors, as specimens from both clades were even present in the same grab samples.

### *Comparative morphology of the two Eastern Atlantic* C. nitidulum *subclades*

Our comparisons of the hard-part morphology (sclerites and radula) between representatives of the two Eastern Atlantic *C. nitidulum* subclades Ia and Ib did not result in any significant differences between specimens of comparable size and similar geographical origins. For example, we analysed four specimens that were found

in the same sample from the Swedish west coast. While the animals looked almost identical, they still clustered in two separate subclades. We cannot exclude, though, that there might be morphological differences between these subclades in soft-body anatomy. Specimens fixed for molecular analyses are not well preserved for histological examination and our specimens were also used for radula extraction, tissue samples for sclerite and DNA extraction. A future study targeting this question by fixing specimens specifically for histology after having taken a small tissue sample for DNA analysis would be desirable.

### *Ontogenetic and geographical variations in* C. nitidulum *s. l. sclerite morphology*

Comparing sclerite morphologies between specimens from different geographical areas, we found distinct differences between specimens of *C. nitidulum s. l.* from the southern and northern areas of the distribution. We thus confirm and broaden the results of Salvini-Plawen (1978), who showed that specimens from the Swedish west coast and from Trondheimsfjord, Norway, differed slightly but distinctly in sclerite morphology. The geographical differences are even more distinct when including specimens from northern Norway (Porsangerfjord, Finnmark) and Svalbard. The variation in sclerite morphology within *C. nitidulum s. l.* found here is larger than that previously described in this taxon. Comparison of specimens from a larger sampling range than previous studies has revealed that specimens of *C. nitidulum* from the northern part of the extension range have a relatively consistent sclerite morphology, while specimens from the southern part show larger variations in sclerite morphologies. These morphologies do not correspond to the two molecular clades found in the area.

Our comparison of sclerite morphology in specimens of different sizes revealed ontogenetic changes. This is particularly evident when comparing juvenile and adult specimens. This morphological variation related to ontogeny can obscure taxonomic relationships; therefore, when comparing sclerite morphology between specimens for taxonomic purposes, it is advisable to make comparisons using specimens of similar sizes.

### *Radula morphology in* C. nitidulum*—ontogenetic change from a* Falcidens*- to a* Chaetoderma*-type radula*

The radula of small *C. nitidulum s. l.* specimens looks very similar to a typical *Falcidens*-type radula (*sensu* Scheltema, 1972), which is defined by a pair of sickle-shaped teeth attached to the radula cone and a sclerotized plate. A prominent difference between the radula of *Falcidens* as described by Scheltema (1972, 1981) and the juvenile *C. nitidulum s. l.* radula is the lack of a sclerotized symphysis between the teeth of the latter. Our own unpublished observations on *F. crossotus* show that the symphysis here is short and well defined, and appears to be quite flexible. It is medially attached to the posterior (ventral) surface of the tip of the cone.

The close similarity between the juvenile *C. nitidulum* radula and a *Falcidens*-type radula means that caution has to be employed when describing *Falcidens* species from few and small individuals. It is likely that some species assigned to *Falcidens* are in fact juveniles of a *Chaetoderma* species. Among these is *F. sterreri* (Salvini-Plawen, 1967), which was described from Gullmarsfjorden, Sweden, and later recorded also from localities close to Bergen, geographic areas that were included in our study. Our detailed analyses suggest that *F. sterreri* might have been based on *C. nitidulum* juveniles. A similar case might be *F. lipuros* (Scheltema, 1989), from Tasmania, which was described from two small specimens (the larger one 6.8 mm) that showed a very similar radula to the *C. nitidulum* juveniles. A more detailed investigation, including the use of molecular data, is needed to support this suggestion and to find the possibly corresponding *Chaetoderma* species. In addition, it is possible that the specimens described by Ivanov (1981) as *Caudofoveatus* represent a transitory state. All specimens assigned to this genus were close to 10 mm in length and, based on the average adult size of *Chaetoderma* species, could be juveniles. Again, molecular analyses could clarify the status of this genus.

The radula of larger specimens of *C. nitidulum* closely matches the descriptions by Scheltema (1972, 1981) of the typical *Chaetoderma*-type radula, which were based on *C. canadense*. One difference we found was that the cuticular dome in *C. nitidulum* does not surround the tip of the radular cone, but passes behind it and thus is horseshoe-shaped in cross-section (Fig. 9). Scheltema (1972), in contrast, illustrated it as oval in shape and surrounding the cone. SEM micrographs of *C. argenteum* show that in this species the frontal upper margin of the dome is at almost the same level as the posterior margin and that in this case the dome is ring-shaped (Scheltema, Buckland-Nicks & Chia, 1991). Thus, there appear to be some species-specific differences regarding the morphology of the cuticular dome.

### *Implications for chaetodermatid taxonomy and classification*

Our molecular phylogenetic analyses suggest that the radula characters presently used for classification within Chaetodermatidae are ambiguous, because the differences in radula morphology in part represent ontogenetic changes. While in species of *Falcidens* the pincer-like radula is retained throughout life, in *C. nitidulum s. l.* the radula changes from a *Falcidens*-type to a *Chaetoderma*-type radula, with the cuticular parts of the radula increasing in size as the animals grow. The *Falcidens*-type is therefore not an apomorphy for the genus *Falcidens* and the diagnosis of *Chaetoderma* needs to be amended.

It remains to be investigated whether all species of *Chaetoderma* undergo ontogenetic changes in radula morphology, as found in *C. nitidulum s. l.* Radulae from juveniles of *Caudofoveatus* and *Furcillidens* have not been described and so it is unknown if the radula morphology in these two genera changes during ontogeny. In consequence, a revision of the Chaetodermatidae is necessary to evaluate whether the currently recognized genera are valid and to investigate other potential morphological characters that could be used to differentiate between them, especially in their juvenile stages.

Two other *Chaetoderma* species, *C. intermedium* and *C. productum*, occur in the northeastern Atlantic. While distinct morphological differences have been pointed out between *C. nitidulum* and these two species (Salvini-Plawen, 1975, 1978), no genetic data are available. In addition to *C. canadense*, three further *Chaetoderma* species have been described from the northwestern Atlantic: *C. bacillum*, *C. lucidum* and *C. squamosum* (Heath, 1918). Material of these species was not available for investigation and their descriptions are too brief for adequate morphological comparison. The genetic relationships of these species to the other North Atlantic Chaetodermatidae remain to be evaluated.

### *One or many? Is* Chaetoderma nitidulum *a hypervariable species or a species complex?*

While molecular data clearly indicate a separation of *C. nitidulum s. l.* into three distinct genetic lineages, morphological data do not show the same pattern. However, species delimitation is not always possible by morphological means, since speciation is not necessarily accompanied by morphological change (Bickford *et al.*, 2007). It remains to be seen whether the genetic lineages represent distinct species, or represent within-species population structure (Sukumaran & Knowles, 2017). Differences in sclerite and radula characters, which are traditionally considered most important for species delimitation in *Chaetoderma*, are not consistent with our molecular results for the two Eastern Atlantic subclades Ia and Ib. All specimens of the Western Atlantic clade II that were available for the study were juveniles. Therefore we refrain from defining a new species and giving a

morphological diagnosis for this clade. Investigation of additional morphological characters from representatives of the three *C. nitidulum s. l.* clades is needed.

At present, we recommend that *C. nitidulum s. l.* is treated as one species and that the name *C. nitidulum* should be used for all North Atlantic *Chaetoderma* with striated trunk sclerites. We do not believe that molecular data alone justify the splitting of *C. nitidulum* into three separate species. Assignment of separate species names to the Eastern Atlantic molecular subclades is, furthermore, complicated by the fact that specimens of both subclades occur at the type locality and that the type material is not viable for molecular analysis.

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