A European nudibranch new to the Gulf of Maine: Doris pseudoargus Rapp, 1827.

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Abstract

The Gulf of Maine has seen an increasing number of introduced species, some of which have had significant impacts on benthic community structure. In 2017, a number of specimens of the European dorid nudibranch, *Doris pseudoargus*, were observed on rocky ledges in waters off Cape Ann, Massachusetts. The presence of numerous specimens and egg masses suggested that the species had been established before 2017. Subsequent observations and collections have discovered specimens ranging from sites off Wedgeport, Nova Scotia to the northern end of the Cape Cod Canal. Genetic analysis has confirmed the species as *Doris pseudoargus* and similar to specimens from European locations.

Historically, the Gulf of Maine had only one species of sponge feeding nudibranch. Unlike this endemic, stenotrophic feeding dorid, the introduction of a large sized, sponge predator known to feed on diverse sponge species has the potential to significantly impact community structure, as evidenced on vertical rocky substrates where its natural history has been observed.

Introduction

Introduced species are a continuing and growing issue as global trade expands (Elton, 1956; Simberloff, 2013). The Gulf of Maine (GOM) has experienced a number of introductions of both animals (Berman et al. 1991; Dijkstra et al. 2007; Harris and Mathieson 2000) and marine plants (Dijkstra et al, 2017; Mathieson et al. 2003), some of which have become conspicuous members of marine communities (Harris and Tyrrell 2001; Pederson et al. 2005). One group of marine mollusks for which there is little information are members of the Nudibranchia. The diversity of nudibranch species in the GOM, and the Atlantic Maritime Province is significantly lower than that of comparable regions in Europe (for GOM: Bleakney 1996; Moore 1964; Shine 2012; for Europe: Picton and Morrow 1996; Thompson and Brown 1976; Thompson and Brown 1984). However, most of the known GOM species are also found in Europe. Thus, it is very difficult to determine if this is due to amphiatlantic distributions or whether some of the species are cryptic introductions from long-established trade routes between Europe and North America (Simberloff 2013).

Until recently, the only documented introduction of a European nudibranch species to the GOM, was the tritonid, *Candiella plebeia* (Johnston 1828) (as *Tritonia*). It appeared in the early 1980's and then rapidly disappeared within a few years (Allmon and Sebens 1988). A population of *C. plebeia* has been discovered in Eastport, Maine in recent years (Harris et al. manuscript in preparation). A unique feature of the nudibranch fauna of the GOM has been the lack of dorid species that prey on sponges. Historically, only one species, *Cadlina laevis* (Linnaeus 1767), which preys on *Halisarca* sp. was known from the GOM. In Europe however, there are a number of sponge-eating dorid species (Thompson and Brown 1984), that is much more typical of marine communities in many regions of the world (Behrens and Hermosillo 2005; Chavanich et al. 2010; Coleman et al. 2015; Debelius and Kuiter 2007; Gosliner 1987; Gosliner et al. 2008; Valdes et al. 2006). This long time distribution changed however in 2017, when a population of the large European dorid, *Doris pseudoargus* Rapp 1827, was observed on rocky ledges off Cape Ann, Massachusetts. *D. pseudoargus* is a common sponge feeding dorid nudibranch on European shores whose wide distribution extends from the Mediterranean Sea to the northern European coastline (Miller 1961;
The species feeds on a variety of sponges, although most references cite *Halichondria panicea* (Pallas 1766) as a dominant prey item (Thompson and Brown 1984; Van Bragt 2004). *H. panicea* commonly occurs in the Gulf of Maine (Hartman 1964; Martinez 2010; Minor 1940; Pollock 1997).

The purpose of this study is to describe the information obtained and analyzed since that initial observation in 2017. The ecological implications of this introduction are also discussed.

**Materials And Methods**

Observations from a series of exploratory dives were made in the region of Cape Ann, Massachusetts by A. Shure. Photographs of individuals and its habitat were taken along with notes on observations, depth and associated fauna. On July 26, 2017, four live specimens were collected and taken to the University of New Hampshire (UNH), Durham, New Hampshire and maintained in a recirculating sea water system. The live specimens were photographed and then preserved in 95% EtOH. Two preserved specimens were deposited at the California Academy of Sciences, San Francisco, California and one was used for genetic analysis. Genetic comparissons with European specimens of *Doris pseudoargus* confirmed the Massachusetts specimen's identity. Additional observations and collections have since been made by A. Shure and others and are included. Literature searches were also conducted to determine whether additional specimens had been observed and reported at other locations in the GOM. The information obtained is synthesized below.

**Genetic Taxon Sampling**

A 658 bp region of the mitochondrial gene cytochrome oxidase I (COI) was sequenced for one Massachusetts *D. pseudoargus* specimen (CASIZ 223159A) and submitted to GenBank with the following accession number: MZ389059. Genetic comparisons were made with available GenBank material including the following seven sequences of European *D. pseudoargus* collected from the North Sea: one specimen from Esjerg, Denmark (KR084907); two specimens from Heligoland, Germany (KR084616 & KR084586); one specimen from Inverness, Scotland (KR084378); one specimen from Kingsbarns, Scotland (AY345030); one specimen from Kattegat, Sweden (MG935320); and one specimen from Skagerrak, Sweden (MG935407). Outgroup comparisions included *Doris ocelligera* (Bergh 1881) and *Doris adrianae* Urgorri and Señarís 2021 from the Mediterranean and *Doris montereyensis* J.G. Cooper 1863 from the Eastern Pacific.

**DNA Extraction, Amplification, and Sequencing**

DNA extraction was performed on a small tissue sample from CASIZ 223159A using the Qiagen Dneasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) spin column extraction method. Polymerase chain reaction (PCR) was used to amplify a 658 bp fragment of COI using Folmer et al. (1994) universal primers (HCO2198: 5′-TAAACTTCAAGGGAGATGTCTTAA-3′; LCO1490: 5′-GGTCAACAATCTATAGACATATTGG-3′). The reaction mixture contained the following: 2.5 μL of 10× PCR buffer, 0.5 μL dNTPs (10mM stock), 0.5 μL of each primer (10μM stock), 0.25 μL DreamTaq™ Hot Start DNA Polymerase (5U/μL, Thermo Fisher), 5 μL betaine, 2 μL bovine serum albumin (BSA), 4 μL of template DNA,
and then filled to a final volume of 25 μL with Millipore-H2O. The following PCR protocol was run on a BioRad MyCycler Thermocycler (Bio-Rad Laboratories) at the California Academy of Sciences Center for Comparative Genomics (CCG): an initial denaturing for 3 min at 94 °C, followed by 40 cycles of denaturing for 30 s at 94 °C, annealing for 30 s at 46 °C and extension for 45 s at 72°C with a final extension period of 10 min at 72 °C. Amplified DNA was stained with ethidium bromide and examined using gel electrophoresis on a 1% TBE agarose gel. Successfully amplified products were cleaned using an ExoSAP-IT protocol (USB Scientific) before being sequenced at ELIM Biopharmaceuticals (Hayward, CA, USA).

**Data analysis**

Successfully sequenced fragments were assembled, trimmed to remove primers, and edited using Geneious v11.1.5 (Kearse et al. 2012) and Mesquite v3.61 (Maddison and Maddison 2018) before alignment with MAFFT (Katoh et al. 2009). Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were used to estimate the evolutionary relationships within *D. pseudoargus*. Best-fit evolution model partition definitions for BI and ML analyses were determined for each codon position in COI using ModelTest-NG (Darriba et al. 2020; Flouri et al. 2014) on XSEDE via the online CIPRES Science Gateway (Miller et al. 2010). The following best-fit evolution models were applied for the Bayesian inference analysis: SYM+I for codon position 1, F81+I for codon position 2, and HKY+G for codon position 3. Bayesian inference was performed in MrBayes v3.2.7a (Ronquist and Huelsenbeck 2003) and the dataset was run for $2.5 \times 10^7$ generations. Markov chains were sampled every 1000 generations and the standard 25% burn-in calculated before a 50% majority rule consensus tree of calculated posterior probabilities (pp) was created from the remaining tree estimates. Maximum likelihood was performed using randomized accelerated maximum likelihood (RAxML) v8.2.12 (Stamatakis 2014) and non-parametric bootstrap values (bs) were estimated from $2.5 \times 10^4$ fast bootstrap runs set with the evolution model GTR+GAMMA. Tree branches were considered strongly supported if $pp \geq 0.95$ and $bs \geq 70$, while $pp \leq 0.94$ and $bs \leq 70$ were considered to have low support (Alfaro et al. 2003).

A TCS haplotype network based on an ingroup COI alignment of *D. pseudoargus* with the connection limit set to 95% (v1.21; Clement et al. 2002) was constructed using PopArt v1.7.2 (Leigh and Bryant 2015). Automatic Barcode Gap Discovery (ABGD) analysis outlined by Puillandre et al. (2012) was used to detect genetic variation within *D. pseudoargus*. An ingroup COI alignment was uploaded to the ABGD Web-based interface (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) and the following parameters were applied: Jukes-Cantor (JC69) $P_{\text{min}} = 0.001$, $P_{\text{max}} = 0.2$, Steps = 10, NB = 20 with a $x = 1.3$ relative gap width.

**Data availability**

Additional information on specific observations and collecting sites can be obtained by directly contacting the following web site and individual authors. For the Nova Scotia report see https://www.inaturalist.org/observations/70940660. A. Kuzirian (akuzirian@mbl.edu) has specific information on the intertidal sites at Scusset Beach and A. Shure (Alex.Shure@gmail.com) has detailed information on observations and specimens collected at subtidal sites in Massachusetts. L. Harris
(larry.harris@unh.edu) can provide details on observations and specimens collected at the Isles of Shoals and Cape Neddick. T. Gosliner (tgosliner@calacademy.org) can respond to inquiries concerning the genetic analyses.

**Results And Observations**

In 2017, a population of *Doris pseudoargus* was observed on subtidal rocky ledges at Paddock Rock off Cape Ann, Massachusetts by A. Shure. All animals occurred at a depth below 13m. There were a number of large animals and egg masses that suggested the species had a stable and well established population (Fig. 1). The animals were feeding on the sponge *Isodictya palmata* (Ellis and Solander 1786) as is evidenced by the feeding wound in the image (Fig. 1a). A large egg mass, a partial one, and a pair of *D. pseudoargus* mating in the background are visible in the image (Fig. 1b). However, a survey of photographic images from fall 2016 taken by Shure at the same location did not show any specimens present.

**Fig. 1** Images of *Doris pseudoargus* taken at Paddock Rock by A. Shure. **Fig 1a** large individual of *D. pseudoargus* crawling on natural substrate. Colony of the sponge *Isodictya palmata* shows evidence of grazing by nudibranch (arrow). **Fig 1b** two egg masses (asterisks) of *D. pseudoargus* on rock ledge; one complete, one partial. A pair of mating *D. pseudoargus* is visible in background (arrow) among colonies of the sponge *I. palmata*. Gloved fingers of diver provide scale; animals and egg masses are larger than endemic Gulf of Maine dorid nudibranch species.

Subsequent dives by Shure recorded specimens at a series of additional sites in the GOM, including Halfway Rock and the wreck of the ship/vessel Chester Poling (Table 1). The nudibranchs at most of the deeper sites were observed feeding on *I. palmata*. Additional specimens were also observed at several locations in 2018 through 2021 (Table 1). In each case the habitat consisted of rocky ledges below the thermocline that supported populations of sponges, particularly the palmate sponge, *I. palmata*. The only time specimens were observed on artificial substrates was on the wreck of the Chester Poling. Over time, Shure has noticed that there appears to be a decline in the abundance of *I. palmata* at the dive sites compared to earlier years. In 2020, Shure observed specimens of *D. psuedoargus* at Graves Light Station, that occurred at depths as shallow as 4m and were feeding on *Halichondria panicea* (Table 1). Captain J. Sullivan of the charter vessel Keep-ah, told Shure in 2020 that there had been specimens observed at Graves Light Station for the past couple of years.

**Table 1.** Summary of NW Atlantic observations including dates, locations, relative numbers and egg masses when present.
* 4 specimens collected and transported to University of New Hampshire

**Fig. 2** Map of Massachusetts and New Hampshire coastal zone showing location of dive sites indicated by letters annotated in Table 1.

In June, 2019, three specimens were collected off Broad Cove of Appledore Island, Isles of Shoals, ME by divers from the Shoals Marine Laboratory. J. Factor then preserved the animals and delivered them to Harris at UNH. A live specimen was collected at the same site by E. Kintzing and brought to Harris and kept in a recirculating sea water system, photographed and preserved in 95% EtOH. Additional dives in the area reported more specimens present, including egg masses. In November, 2019 and 2020, a breeding population of numerous individuals was observed by the University of New Hampshire Advanced Diving Class at Mingo Rock, a subtidal ledge north of Appledore Island.

In April, 2021, a specimen was observed but not photographed by E. Kintzing on a dive at Cape Neddick, York Beach, Maine. The identification as *D. pseudoargus* was confirmed by size and morphology (much larger than any native dorid nudibranchs known for the GOM and the observer was familiar with the specimens observed at Appledore Island and Mingo Rock). An identifiable egg mass was observed and photographed at the same site at Cape Neddick a couple of weeks later though the nudibranch was not seen.
In 2019, A. Kuzirian, observed a smaller specimen of *D. pseudoargus* collected by Marine Biological Laboratory, Woods Hole, MA collectors from the northern end of the Cape Cod Canal. It was photographed and subsequently preserved for genetic analysis.

Recently, A. Shure discovered a web sighting of what is likely to be *D. pseudoargus* collected off Yarmouth County, Nova Scotia, Canada in 2017 (https://www.inaturalist.org/observations/70940660). This sighting would indicate that the species was already widely distributed when it was first observed off Cape Ann. However, the more recent sightings at the Isles of Shoals and Cape Neddick suggest the species is fairly recent and is expanding its range southward.

On September 27, 2021, W. Grossman, a MBL collector, found three specimens under rocks at an intertidal site at Scusset Beach at the north end of the Cape Cod Canal. The specimens were found coincident with populations of the sea anemone, *Metridium senile*. However, very few sponges were observed in the vicinity.

A somewhat unique feature of *Doris psuedoargus* compared to most European nudibranch species is the fact it occurs in a wide range of color patterns, including mottled browns (Harris, personal observations; Thompson and Brown 1984). The specimens photographed by Shure and specimens brought to Spaulding Hall for preservation ranged from somewhat uniform colors of yellow and orange (collected specimens) to more expanded color ranges including mottled brown (Fig. 1a & b; field photographs by Shure).

**Genetic analyses**

Our Bayesian and maximum likelihood analyses (Fig. 3a) reveal that the specimen sequenced from Massachusetts (CASIZ 223159A) is nested within a well-supported (pp = 1, bs = 97) cluster of European specimens clearly identifiable as *Doris pseudoargus*. Within this cluster of *D. pseudoargus*, there is no clear geographical separation between the European populations and the relationships between all specimens are tentative due to low Bayesian internal node support and the resulting polytomies. The specimen from Massachusetts (MZ389059) groups together with specimens from the North Sea of Scotland (KR084378 & AY345030) and one individual from Heligoland, Germany (KR084586) and Kattegat, Sweden (MG935320) in a weakly supported polytomy; however, this relationship does correspond with the relationships suggested in the TCS haplotype network (Fig. 3b).

The TCS haplotype network of eight specimens of *D. pseudoargus* suggest seven unique haplotypes with no geographical separation and one shared haplotype between a specimen from Inverness, Scotland (KR084378) and one of two specimens from Heligoland, Germany (KR084586). It also suggests that the Massachusetts specimen, the four European specimens from the shared polytomy, and the specimen from Skagerrak, Sweden (MG935407) are more closely related than the specimen from Esjerg, Denmark (KR084907) and the second specimen from Heligoland, Germany (KR084616).

The ABGD analysis of the mitochondrial COI gene also supports the relationships suggested in the phylogenetic analyses and the TCS haplotype network (Fig. 3b). The Massachusetts specimen has lower
genetic variation (0.15-0.31 %) with the five more closely related European specimens (KR084378, AY345030, KR084586, MG935320, MG935407) suggested in the TCS haplotype network. In contrast, there is larger variation (0.76 % in both instances) between the Massachusetts specimen and the specimen from Denmark (KR084907) and the specimen from Heligoland, Germany (KR084586). Since the specimens within European waters exhibit greater genetic variation between themselves (0.0-0.92 %) than to the specimen from the western Atlantic, it strongly supports the hypothesis that the Massachusetts specimen indeed represents an introduction from Europe.

**Fig. 3** A. Bayesian phylogenetic tree of *D. pseudoargus* estimated from COI data. Numbers above / below branches refer to BI posterior probabilities (pp) / ML non-parametric bootstrapping values (bs). Relationships not recovered in ML analysis indicated by a dash. Different colors represent geographical locations. B. TCS haplotype network for *D. pseudoargus* COI. Each circle indicates a unique haplotype, size indicates number of specimens sharing that haplotype, lines between circles indicates a single substitution, small black circles indicate hypothetical haplotypes, and colors correspond to geographical locations in A.

**Discussion**

*Doris pseudoargus* is a common species in European waters and occurs from the intertidal to deeper habitats down to 300m (Thompson and Brown 1984). The species feeds on several different sponge species in Europe, including *Halichondria panicea*, which is common in the Gulf of Maine (GOM). The initial GOM discovery and most subsequent observations of the species have been from deeper subtidal locations (> 15m) on rocky ledges (Table 1). Specimens have subsequently been found at shallower depths including 4m at Graves Light Station and intertidally in the Cape Cod Canal (Table 1). The sighting in Nova Scotia (https://www.Inaturalist.org/observations/70940660) did not include depth information and the specimens observed at Lanes Cove and Cape Neddick were shallower (Table 1). It is interesting that it was not observed at Paddock Rock in 2016 while numerous breeding specimens were observed in June 2017. It is likely that recruitment may have occurred, but individuals were small, cryptic and not seen. The fact that it was observed in Nova Scotia as well as off Cape Ann in the southern portion of the Gulf of Maine suggests it was present and became established and spread without being detected for some time prior to 2017. The increasing popularity of underwater photography and citizen science increases the likelihood that new observations will be detected and recorded by persons who are informed enough to realize a species may not be typical of that habitat. The presence of specialized photo guides (Martinez 2010; Shine 2012) available to the public also increases the likelihood of new or introduced species being sighted. While the rediscovery of *C. plebeia* was made by a scientist (Harris) who studies nudibranchs, the discovery of *D. pseudoargus* was by an informed recreational diver (Shure).

The geographic range of *D. pseudoargus* extends from the Mediterranean Sea to the North Atlantic; this implies that the species is able to tolerate a wide temperature range (eurhythmic). Individuals feed on sponges, including *Halichondria panicea* that occur over a wide range of depths from the intertidal to more than 60m. The fact that *D. pseudoargus* had only been found at depths below 13m at most sites until recently (intertidal in the Cape Cod Canal and 4m off Cape Ann and 5m at Cape Neddick) is interesting and is probably why it was not detected earlier. However, the fact that a specimen was likely collected on the
outer coast of Nova Scotia in 2017, where summer water temperatures can be warmer than those found in most of the GOM, begs the question of where it was originally introduced and when.

The size of the initial population observed off Cape Ann suggested that it had been established in the region for some time since there were a range of sizes, including spawning adults. In Europe, *D. pseudoargus* is known to have an annual life cycle which includes settling in the later summer and growing through the winter and then spawning in early summer (Thompson, 1976). This is a similar pattern to that seen for many of the larger nudibranchs occurring in the GOM (Harris, 1973). However, climate change may now be impacting seasonal life history patterns (Pershing et al. 2015; Dijkstra et al. 2017). The observations of *D. pseudoargus* in the GOM have documented large animals mating and producing egg masses in November through June, suggesting a more extended reproductive cycle. It is possible that the initial distribution in deeper, more stable (colder) water masses may have resulted in a breakdown in the seasonal reproductive pattern. It begs the question about what other species may be present in the Gulf of Maine, but at depths deeper than normally explored by divers. Bleakney (1996) lists nudibranch species reported for Nova Scotia, which have not been documented in the GOM, so it is quite possible that new discoveries are likely as more divers become aware of the possibility of finding additional species.

The introduction of a large sponge predator that has been absent from the Gulf of Maine and the Canadian Maritimes, introduces the issue of its potential impact on the local sponge fauna that historically has had very limited predation pressure. In shallower depths, only the sea star *Henricia sanguinolenta* (O.F. Müller 1776) has been documented as a sponge predator (Dijkstra et al. 2013; Sheild 1990; Sheild and Witman 1993; Van Volkom et al. 2021). The documented species that *D. pseudoargus* preys upon in the Gulf of Maine have become less common in shallow waters. This reduction in abundance has been linked to space competition/availability by a series of introduced colonial ascidians (Dijkstra et al. 2007; Dijkstra and Harris 2009). At Paddock Rock, sponge populations do appear to have declined after the presence of large numbers of *D. pseudoargus* predators (Shure, unpublished observations) and it is likely to be the case with Mingo Rock with numerous individuals present two years in a row. Future directed studies are needed to understand the impact of nudibranch predation on community structure on vertical walls that typically are dominated by suspension feeding, colonial invertebrates of which sponges are an important component.

**Declarations**

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This project was not directly supported by outside funding sources. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript. J. Factor and W. Grossman provided specimens collected off Appledore Island and Scusset, MA respectively. E. Kintzing collected one specimen and provided observational information from the Isles of Shoals and Cape Neddick. Live specimens were maintained for observation in the aquatic room in Spaulding Hall, University of New Hampshire and genetic analyses were conducted at the California Academy of Sciences. Cape Ann
offshore transportation and dive logistics were provided by Dave Stillman, Mary Stillman, Steve Smith, and David Shumway.

References


39. Sheild CJ (1990) Predation by the sea star Henricia sanguinolenta (Echinodermata: Asteroidea), with special attention to its effect on sponges. Masters Thesis. Northeastern University, Boston, USA


41. Shine J (2012) Nudibranchs of the Northeast. Blue Sphere Pubs, China


Figures

Figure 1

Images of *Doris pseudoargus* taken at Paddock Rock by A. Shure. **Fig 1a** large individual of *D. pseudoargus* crawling on natural substrate. Colony of the sponge *Isodictya palmata* shows evidence of grazing by nudibranch (arrow). **Fig 1b** two egg masses (asterisks) of *D. pseudoargus* on rock ledge; one complete, one partial. A pair of mating *D. pseudoargus* is visible in background (arrow) among colonies of the sponge *I. palmata*. Gloved fingers of diver provide scale; animals and egg masses are larger than endemic Gulf of Maine dorid nudibranch species.

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Map of Massachusetts and New Hampshire coastal zone showing location of dive sites indicated by letters annotated in Table 1.

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