Taxonomic and biological findings on thyasirid bivalves from the Canadian Arctic and Eastern Canada

by

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Abstract

The Thyasiridae are small marine clams common in cold waters, including around the Canadian Arctic and Eastern Canada. Some thyasirid species form nutritional symbioses with sulfur-oxidizing bacteria and may act as ecosystem engineers in sediments recovering from organic enrichment. Using thyasirids as ecological indicators requires accurate species identification, and there is a lack of taxonomic attention to thyasirid species across large geographic areas. This thesis aims to improve our understanding and ability to identify 5 thyasirid species (*Thyasira* cf. *dunbari, T.* cf. *equalis, T. plana, T.* cf. *gouldi,* and *Axinopisda orbiculata*) occurring in the Canadian Arctic and Eastern Canada, based on the study of multiple characters (anatomical, genetic, and symbiotic). Examination of the recently discovered *T.* cf. *gouldi* complex led to the reinstatement of a formerly synonymized species, *T. plana.* Chemosymbiosis is confirmed for the first time in arctic thyasirids, namely in *T.* cf. *gouldi*; other thyasirids from this region are asymbiotic. New taxonomically informative gene sequences are presented, and a re-examination of the distribution of thyasirid species notably revealed *T.* cf. *dunbari* occurrences at lower latitudes than *T. dunbari*. Future work is needed to improve the taxonomy of thyasirids and to inform their usefulness as indicators of organic enrichment.

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List of Abbreviations and Symbols

Anatomical abbreviations (Oliver and Killeen, 2002):

aa - anterior adductor muscle

agr - anterior glandular ridge

ct - ctenidium

dg - lateral body pouch

f - foot

id - inner demibranch

lbp - lateral body pouch

lp - labial palps

od - outer demibranch

pa - posterior adductor muscle

pgr - posterior glandular ridge

ppr - posterior pedal retractor muscle

r - rectum

Institutional abbreviations:

CMN - Canadian Museum of Nature

MCZ - Museum of Comparative Zoology

NFM - The Rooms Corporation of Newfoundland and Labrador

WoRMS - World Register of Marine Species

USNM - Smithsonian National Museum of Natural History

USFC - United States Fish Commission

Other:

µm - Micrometer

˚C - degrees Celsius

cm - Centimeter

FEI - Field Electron and Ion Company

g - Gram

m - Metre

mm - Millimetre

NL - Newfoundland and Labrador

OTU - Operational taxonomic unit

TEM - Transmission electron microscopy

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Chapter 1: Introduction

1.1 The bivalve family Thyasiridae and chemosymbiosis

The bivalve family Thyasiridae (superfamily Thyasiroidea, Taylor *et al.* 2007) is globally distributed in cold marine waters, occurring infaunally within various types of substrates (e.g., mud, sand) from shallow to abyssal depths (Payne & Allen 1991). Thyasirds are small clams (generally less than 10 mm long, with notable exceptions such as the genus *Conchocele* that reaches 18 cm; Kharlamenko *et al*. 2016) that often form dense populations in organic matter-rich sedimentary environments (e.g. whale falls, fjords, cold seeps, sedimented hydrothermal vents, oil spills, sewage outfalls, aquaculture sites; Dando & Southward 1986; Dando & Spiro 1993; Oliver & Killeen 2002; Oliver & Sellanes 2005; Oliver & Holmes 2006; Carlier *et al.* 2010). The family has received particular interest because they are one of eight bivalve families that includes both asymbiotic and symbiotic species, and because the latter maintain bacteria at the external surface of gill epithelial cells (Dando & Southward 1986; Southward 1986; Dufour 2005; Oliver & Rodrigues 2017), in all but one species (Fujiwara *et al.* 2001). The Thyasiridae are a diverse and widespread family within the chemosymbiotic bivalves; in this family, chemosymbiotic species occur in multiple clades, sometimes within the same clades as asymbiotic species (Taylor *et al.* 2007). In addition, these symbiotic and asymbiotic species co-occur, as shown in the Bonne Bay fjord in Newfoundland, Canada (Batstone *et al*. 2014; Zanzerl *et al*. 2019).

Many thyasirids are mixotrophic and nutrient-dependent on both their sulphide-oxidizing bacterial symbionts and on particulate food obtained through suspension feeding (Dufour & Felbeck 2006; van der Geest *et al.* 2014) or deposit feeding (Zanzerl *et al.* 2019). Species differ in their degree of nutritional dependence on chemosymbiotic bacteria, partly in accordance with the availability of sedimentary sulphides, but also on the availability of suspended food particles (Dando & Spiro 1993; Dufour & Felbeck 2006; Zanzerl *et al*. 2019). Thyasirids that harbour chemosynthetic bacterial symbionts have variably modified gill filaments. Thyasirids have five different gill types, identified as 1, 2, and 3 in Dufour (2005) and 4 and 5 in Oliver (2014). Most symbiotic thyasirid species display type 3 gill filament morphology, including *T. flexuosa*, *T. sarsi* and *T. gouldi*, in which expansion and thickening at their abfrontal end increases the area available for bacterial colonization (Reid & Brand 1986; Dando & Southward 1986; Diouris *et al*. 1988; Distel 1998; Dufour 2005; Batstone *et al*. 2014). In addition, the *Conchocele* and *Ochetoctena* have “tubular/lamellar" (type 4) and “tubular” (type 5) gill types, which are more complex when comparing to the type 3 gill filament morphology (Oliver 2014). Other thyasirid species, such as *Axinopsida orbiculata,* lack chemoautotrophic symbionts altogether and show no gill filament elongation (type 1 and 2 gills) (Dando & Southward 1986; Southward 1986; Dufour 2005).

1.2 Ecological importance through sediment burrowing behaviours

Thyasirids live buried in mud or muddy sand where at least some species make three-dimensional burrow systems, radiating down and outwards from their shell (Allen 1958; Dando & Southward 1986; Dufour & Felbeck 2006; Zanzerl & Dufour 2017). Thyasirids frequently

inhabit organically enriched seafloor environments with enhanced activity of sulphate-reducing bacteria, leading to decreases in the concentration of ambient oxygen and increase in sulphides (Westrich & Berner 1984; Faulwetter *et al*. 2013). In sediments, individuals orient the shell with the umbo uppermost. Thyasirids use their vermiform foot to form burrows in sediments (as seen in Fig. 1.1) to access sulfides from deeper sediments for their symbionts or to deposit feed (Dando & Southward 1986; Dufour & Felbeck 2003; Zanzerl & Dufour 2017; Zanzerl *et al*. 2019). The foot can extend up to 30 times the length of their shell (Dufour & Felbeck 2003). They also use their foot to form a semi-permanent inhalant tube that reaches the sediment surface, providing access to oxygenated water as well suspended food (Allen 1958). In some species, their foot also builds an exhalent tube, which often ends in a small chimney projecting above the sediment surface (Oliver & Killeen 2002). Water flowing into the mantle cavity brings oxygen and reduced sulfur to the bacteria held within the gills, and, for both asymbiotic and mixotrophic species, suspended particulate food. Burrow irrigation may enhance sediment oxygenation and thereby promote the recolonization of sediments by macrofauna following organic enrichment events (Dando *et al.* 2004). As such, some thyasirid species could potentially serve as valuable indicators of organic enrichment and/or its recovery within sediments (Oliver & Killeen 2002; Dando *et al.* 2004; Taylor & Glover 2010). To determine properly the ecological function of thyasirids in their environment requires accurate identification of species, characterization of biological traits, and correct documentation of their distribution.

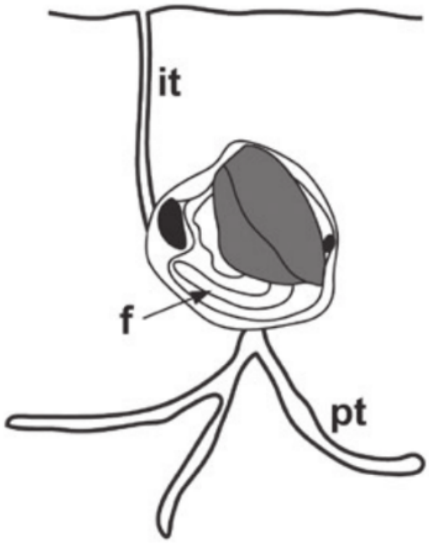

Figure 4.1. An illustration of the burrow system of a thyasirid, showing the location of the inhalant tube (it) and pedal tracts (pt), both formed by the foot (f), as shown in Zanzerl & Dufour (2017). Image reproduced with permission from the Journal of Conchology.

1.3 Taxonomy of thyasirids

Experts consider the Thyasiridae to be a taxonomically challenging group. According to the World Register of Marine Species (WoRMS Editorial Board 2023), the family includes 22 genera, two of which have alternate accepted names. Assignment of taxa within genera can be problematic and WoRMS listings include numerous unaccepted and synonymized names, often reflecting changes in the generic placement of species. Within the largest genus, *Thyasira*, WoRMS listings comprise 5 subgenera, all of which have been considered by some authors to be distinct genera. For example, *Thyasira (Mendicula) ockelmanni* is accepted as *Mendicula ockelmanni*, signaling uncertainty in the characteristics that define *Thyasira* and *Mendicula* as well as in the relationships between these two taxa.

Thyasirid species identifications are based mainly on shell characters, which are thought to be highly variable within a species (Payne & Allen 1991; Oliver & Killeen 2002; Taylor *et al.* 2007). Use of shell characters is especially concerning given that thyasirid shells are often subtle in expression, making identifications difficult to even an experienced observer. Original descriptions and illustrations are generally spare in detail, with graphical depictions often restricted to simple line drawings of the shell (see Fig. 1.2), particularly for the geographically widespread, common taxa that were described in the 1800s. An example of this is seen in *Thyasira gouldi*, a species described based on shell specimens collected from Massachusetts, by the German malacologist R. A. Philippi (1845). Gould (1841) first identified the specimens examined by Philippi as *Lucina flexuosa*; this species was originally described from British waters as *Tellina flexuosa* Montagu (1803). Like *T. gouldi*, the original cursory description of *L. flexuosa* was based on somewhat vague shell features (Oliver & Killeen 2002). Gould (1841) then considered *L. flexuosa* to occur in both European and American waters, despite Philippi (1841) arguing for the presence of separate species in these two regions based on differences in shell morphology. Gould and Binney (1870) agreed with Philippi and *gouldi* became solely associated with American shell specimens. This discussion caused uncertainty among authors several years later and *T. gouldi* has since been identified in European waters and elsewhere (i.e., Canada, Russia, Greenland, Scotland, Norway, and the Pacific coast) (Miloslavskaja 1970; Ockelmann 1958; Bernard 1983; Oliver & Killeen 2002). Type material of *T. gouldi* and some other species described by Philippi were lost and neotypes have not been designated (Oliver & Killeen 2002).

In addition, some taxa described by Jeffreys remain problematic due to missing type material, most notably for species that were erected without full descriptions, i.e. *T. polygona* (Jeffreys, 1864) and *T. flexuosa* (Warén 1980; Oliver & Killeen 2002). Recently, Huber (2015) located types of *T. rotunda* (Jeffreys, 1881) and Oliver *et al.* (2017) found types of *T. flexuosa* in Exeter Museum. Few *Thyasira* spp. original descriptions and illustrations have been revised, resulting in persistent confusion in identifications of common taxa.

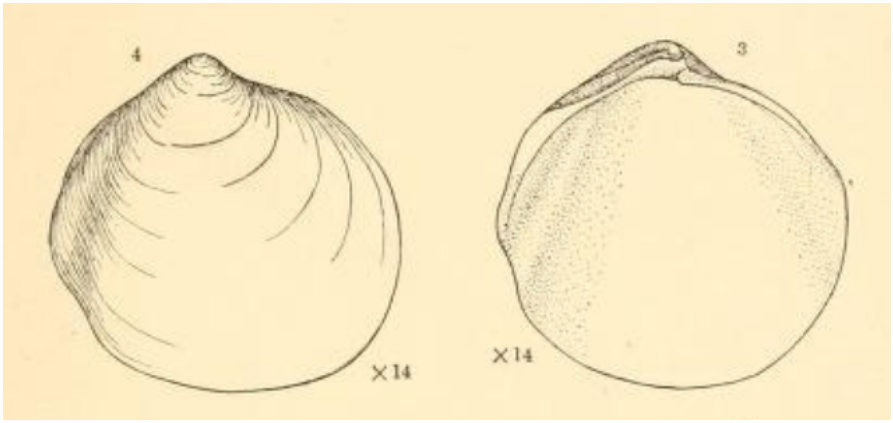

Figure 1.2. Original drawings of *Thyasira plana* from plate 88, figs. 3, 4 in Verrill A.E. & Bush K.J. (1898). Fig. 3 is the interior of the left valve of the type specimen, while fig. 4 shows the exterior of the right valve of the same specimen; X 14. Image from the Biodiversity Heritage Library, contributed by Smithsonian Libraries.

1.4 Thyasirids in Eastern Canada and the Canadian Arctic

In Canadian waters, the identity of thyasirids is poorly known, with most assigned to two "catch-all" species, *Thyasira gouldi* and *Thyasira flexuosa*, which have shells of variable shapes and very broad distributions (Oliver & Killeen 2002). Research on specimens resembling

Thyasira gouldi in Newfoundland has revealed a cryptic species complex of two symbiotic and one asymbiotic operational taxonomic units (OTUs), now identified as *T.* cf. *gouldi* OTUs 1, 2 and 3 (Batstone *et al.* 2014). After examining many specimens at the Canadian Museum of Nature (CMN), we concluded that understanding of the identification of thyasirids in the Canadian Arctic and Eastern Canada remains poor. Noting the absence of a concerted taxonomic effort in these regions, we aim to help fill some of those gaps in this study. We focus on thyasirids most frequently reported in Canadian waters: *Thyasira* cf. *gouldi, Thyasira plana, Thyasira* cf. *dunbari, Thyasira.* cf. *equalis* and *Axinopsida orbiculata*.

1.5 Thesis Objectives

This thesis addresses some of the taxonomic challenges in identifications of thyasirid species found within Eastern Canada and the Canadian Arctic, with the aim of assisting the work of benthic researchers who identify and/or collect thyasirids. The approach used here combines morphological characters and genetic work, and accounts for biogeography.

Chapter 2 investigates the *Thyasira gouldi* complex described in Batstone *et al*. (2014) by analysing and describing the anatomy of two genetically distinct taxa, while reinstating the species *T. plana*. Further, inferred feeding modes and growth rates of these two taxa are discussed based on stable isotope analyses of tissues and shells (Dando & Spiro 1993; Zanzerl *et al.* 2019). Chapter 3 defines and summarizes key characters of five thyasirid species occurring in Eastern Canada and the Canadian Arctic (*Thyasira* cf. *gouldi, T. plana, T.* cf. *dunbari, T.* cf. *equalis* and *Axinopsida orbiculata*), with new measurements, sequences, and some revisions of

their geographic distribution. Information from this thesis can support future marine biodiversity and ecological studies as well as marine habitat assessment and monitoring.

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Co-Authorship Statement

The research described in this thesis was designed and conducted by Rachelle Dove with guidance from Dr. Suzanne Dufour. Rachelle Dove was responsible for the field and laboratory data collection and analysis, with assistance from Dr. Suzanne Dufour. Chapters within were written by Rachelle Dove with intellectual and editorial input by Dr. Suzanne Dufour. In Chapter II, which will be submitted to the American Malacological Bulletin, Bonita McCuaig performed phylogenetic analysis and Manon Giolland collected morphological measurements on thyasirid bivalves from Bonne Bay, NL, both are co-authors of Chapter II. Authorship of thesis chapters will evolve as the manuscripts develop for publication in the primary literature, recognizing contributions appropriately.

Chapter 2: Taxonomic and biological remarks on *Thyasira* cf. *gouldi* (Bivalvia: Thyasiridae) in eastern Canada and reinstatement of the species *Thyasira plana* (Verrill & Bush, 1898).

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2.1 Abstract

The Thyasiridae are small bivalves commonly found in organically enriched sediments in cold marine waters. Within this family, some species form nutritional symbioses with sulfur-oxidizing bacteria. Thyasirid species identification is mainly based upon shell characters that are often considered to display broad intra-specific variation. Recent work that examined multiple characters, including gene sequences and symbiont presence, has revealed a complex of closely-related taxa resembling *Thyasira gouldi* (Philippi, 1845), a species with a presumed pan-arctic distribution, off the coast of Newfoundland, Canada. We investigated the anatomical, genetic, and symbiotic characters of thyasirids resembling *T. gouldi* from Bonne Bay, Newfoundland as well as Saglek Bank and Frobisher Bay in the Canadian Arctic, and designated asymbiotic

members as belonging to a species previously synonymized with *T. gouldi*: *T. plana* (Verrill & Bush, 1898). For the other symbiotic specimens, we retain the designation *T.* cf. *gouldi* until more information (soft anatomy, gene sequences) becomes available for specimens from the type location (*T. gouldi* s.s.). We note overlap in shell shape across *T. plana*, *T.* cf. *gouldi* and *T. gouldi* s.s., although specimens from the (more southerly) type location have a larger prodissoconch. *T.* cf. *gouldi* can attain a larger size and is genetically distinct from the asymbiotic *T. plana*. Both *T.* cf. *gouldi* and *T. plana* appear to reach larger shell sizes and have larger prodissoconchs at higher latitudes. Our findings suggest that *T. gouldi*, as currently defined, represents a complex of multiple species; properly defining this common species across its purported geographic range will require further examination of presumed *T. gouldi* specimens, especially using genetic approaches.

2.2 Introduction

The Thyasiridae (superfamily Thyasiroidea, Taylor *et al.* 2007) are common infaunal bivalves in cold marine waters worldwide; they often form dense populations in organic matter-rich sediments, both undisturbed (e.g. fjords, cold seeps) and impacted by human activities (e.g. oil fields, sewage outfalls, aquaculture sites) (Oliver & Killeen 2002). Notably, they are one of eight bivalve families that have established nutritional symbioses with sulphur-oxidizing, chemoautotrophic bacteria, maintained in association with their gills (Dando & Southward 1986; Dufour 2005; Oliver & Rodrigues 2017). Across the Thyasiridae, species differ in their degree of nutritional dependence on chemosymbiotic bacteria (Dando & Spiro 1993). Symbiotic thyasirid species typically have gill filaments that are expanded and thickened at their abfrontal end,

thereby increasing the area available for bacterial colonization (Distel 1998; Dufour 2005). Other thyasirid species lack chemoautotrophic symbionts altogether and show no gill filament elongation (Dando & Southward, 1986; Southward 1986; Dufour 2005). Some thyasirid species use their vermiform foot to form burrows in sediments to access sulfides for their symbionts or to deposit feed (Dufour & Felbeck 2003; Zanzerl & Dufour 2017; Zanzerl *et al*. 2019). In at least one thyasirid species, *Thyasira sarsi* (Philippi, 1845), considered to be an ecosystem engineer (Dando *et al.* 2004), burrow irrigation enhances sediment oxygenation and promotes recovery after organic enrichment (Dando *et al.* 2004). To properly determine the ecological function of thyasirids in their environment requires accurate identification of species and characterization of their biological traits.

Experts consider the Thyasiridae a taxonomically difficult group, with species identifications based mainly on shell characters that vary considerably (Payne & Allen 1991; Oliver & Killeen 2002; Taylor *et al.* 2007). In the North Atlantic, anatomical and taxonomic descriptions have focused on deep-water species (Payne & Allen 1991), and most taxonomic research on thyasirids from shelf and coastal depths has examined species from the northeast Atlantic (e.g., Killeen & Oliver 2002a, b; Oliver & Killeen 2002; Keuning & Schander 2010; Rodrigues *et al*. 2012; Oliver & Drewery 2014).

One of the more common shallow-water thyasirids identified in Canadian waters is *Thyasira gouldi* (Philippi, 1845), a species described as being symbiotic, with shells of variable shape and a large prodissoconch/larval shell (Oliver & Killeen 2002; Duperron *et al.* 2013). The species was originally described by the German malacologist R.A. Philippi (1845), based on shell specimens sampled from deep water off Massachusetts. Notably, Gould (1841) had

previously described the specimens examined by Philippi as belonging to the species *Lucina flexuosa*, originally described from British waters as *Tellina flexuosa* (Montagu, 1803). Gould (1841) had therefore considered that *L. flexuosa* occurred on both European and American coasts, but Philippi (1841) argued for the presence of separate species in these two regions based on differences in size, contour and lunule depth. Subsequently, Gould and Binney (1870) agreed with Philippi's assessment and the species name *gouldi* became associated with the American shells. In later years, several authors nevertheless identified thyasirids from European waters and elsewhere as *T. gouldi*: most researchers generally consider the species as circumboreal-subarctic in distribution. It has been reported from across subpolar regions of Russia (Miloslavskaja 1970), from Greenland, from multiple North Sea locations (including Scotland and Norway), and from the Pacific coast of North America (Ockelmann 1958; Bernard 1983; Oliver & Killeen 2002). The species name *T. gouldi* has not only become commonly ascribed to thyasirids with large prodissoconchs and a variably sulcate posterior margin from a considerably large geographical range, but has also subsumed two species described from American waters, *T. plana* (Verrill & Bush, 1898) and *T. inaequalis* (Verrill & Bush, 1898), according to Ockelmann (1961).

The only *Thyasira gouldi* material available from the type location (Massachusetts) consists of dry shells, and the holotype appears to have been lost; we are not aware of any depictions of internal anatomy or DNA sequences available from specimens collected around Massachusetts. Ockelmann (1958) and Oliver and Killeen (2002) indicate that *T. gouldi* has a large prodissoconch measuring 205–270 µm, and that prodissoconch size is a key character for differentiating *T. gouldi* from other *Thyasira* species. The prodissoconch size of *T. gouldi* tends to increase with latitude (Ockelmann pers. comm., in Oliver & Killeen 2002).

Recent work has shown that considering multiple characters, including gene sequences, can lead to taxonomic revisions in thyasirids (e.g., Batstone *et al.* 2014). In Newfoundland, specimens with shell characteristics corresponding to *Thyasira gouldi* form a cryptic complex of two symbiotic and one asymbiotic operational taxonomic units (OTUs), identified as *T*. cf. *gouldi* OTUs 1, 2 and 3 (Batstone *et al.* 2014). In July 2016, we collected thyasirids from the Canadian Arctic (Frobisher Bay and Saglek Bank) and found specimens that were genetically identical to the *T.* cf. *gouldi* OTUs originally described in Bonne Bay, Newfoundland (Batstone *et al.* 2014). Here, we present our 18S and 28S rRNA gene analysis, along with a characterization of anatomical features (shell shape, prodissoconch size, internal anatomy) and symbiont presence in specimens from the Canadian Arctic and Bonne Bay. Based on these results, along with a re-consideration of species that Ockelmann (1961) had synonymized with *T. gouldi*, we analyse and describe the soft anatomy of two genetically distinct taxa within what we consider as the *T. gouldi* complex. This complex includes the reinstated, asymbiotic *T. plana* (previously *T*. cf. *gouldi* OTU 3 or "asymbiotic *T*. cf. *gouldi*" in Batstone *et al.* 2014; Zanzerl *et al.* 2019) as well as the symbiotic *T*. cf. *gouldi* (previously *T*. cf. *gouldi* OTUs 1 and 2 or "symbiotic *T*. cf. *gouldi*" in Batstone *et al.* 2014; Dufour *et al*. 2014; Batstone & Dufour 2016; Laurich *et al*. 2015, 2018; Mariño *et al*. 2019; Zanzerl *et al.* 2019; McCuaig *et al*. 2017, 2020) which may be distinct from *T. gouldi* sensu stricto. We compare anatomical and ecological features (i.e., inferred feeding mode and growth rates based on stable isotope analyses of tissues and shells; Dando & Spiro 1993; Zanzerl *et al.* 2019) of specimens of *T*. cf. *gouldi* and *T. plana* across their known distribution, and highlight key traits that can help to differentiate them from each other. For *T. plana*, we compare 2 syntypes from the USNM database to our specimens.

2.3 Materials and Methods

2.3.1 Specimens examined

During the CCGS *Amundsen* leg 2 cruise in July 2016, thyasirids were collected using a 0.25 m^2 box corer from two regions (Fig. 2.1): inner and outer Frobisher Bay (63°38’N, 68°37'W and 62°55’N, 67°05'W, respectively; depth range: 62–610 m), and Saglek Bank (60°18'N, 62°11′W; 275 m depth), a putative hydrocarbon seep (Jauer & Budkewitsch 2010). Specimens were sieved (1 mm mesh) to retrieve thyasirids: 108 were obtained from Frobisher Bay and 26 from Saglek Bank. Seventeen Saglek Bank specimens were placed in a -20°C freezer for stable isotope analyses. Other specimens (N = 43 from Frobisher Bay, N = 7 from Saglek Bank) were dissected and one gill placed in 95% ethanol for subsequent molecular analysis. For 5 of those specimens from Frobisher Bay and 3 from Saglek Bank we preserved the second gill in 2.5% glutaraldehyde buffered with filtered (0.2 µm) seawater (as in Montanaro *et al*. 2016) for morphological analysis. Shells and remaining tissues were preserved in the same glutaraldehyde fixative (N = 55 from Frobisher Bay, N = 7 from Saglek Bank) or 95% ethanol (N = 53 from Frobisher Bay, N = 2 from Saglek Bank).

Other thyasirids used for morphological analysis in this study were collected in 2011 using a Peterson grab (12 cm diameter/sampling depth) from Bonne Bay (49°30′N, 57°55′W; Fig. 2.1), a subarctic fjord in Western Newfoundland (Batstone *et al.* 2014). We also examined and imaged the shells of 13 *Thyasira gouldi* specimens from the type location, "off Massachusetts" (specifically, 25–90 m depth in Cape Cod Bay and Georges Bank), on loan from the Museum of Comparative Zoology, Harvard University (accession numbers 411814, 410580, 411837, 414973, 117016); these individuals are hereafter designated as *T. gouldi* s.s.. Museum

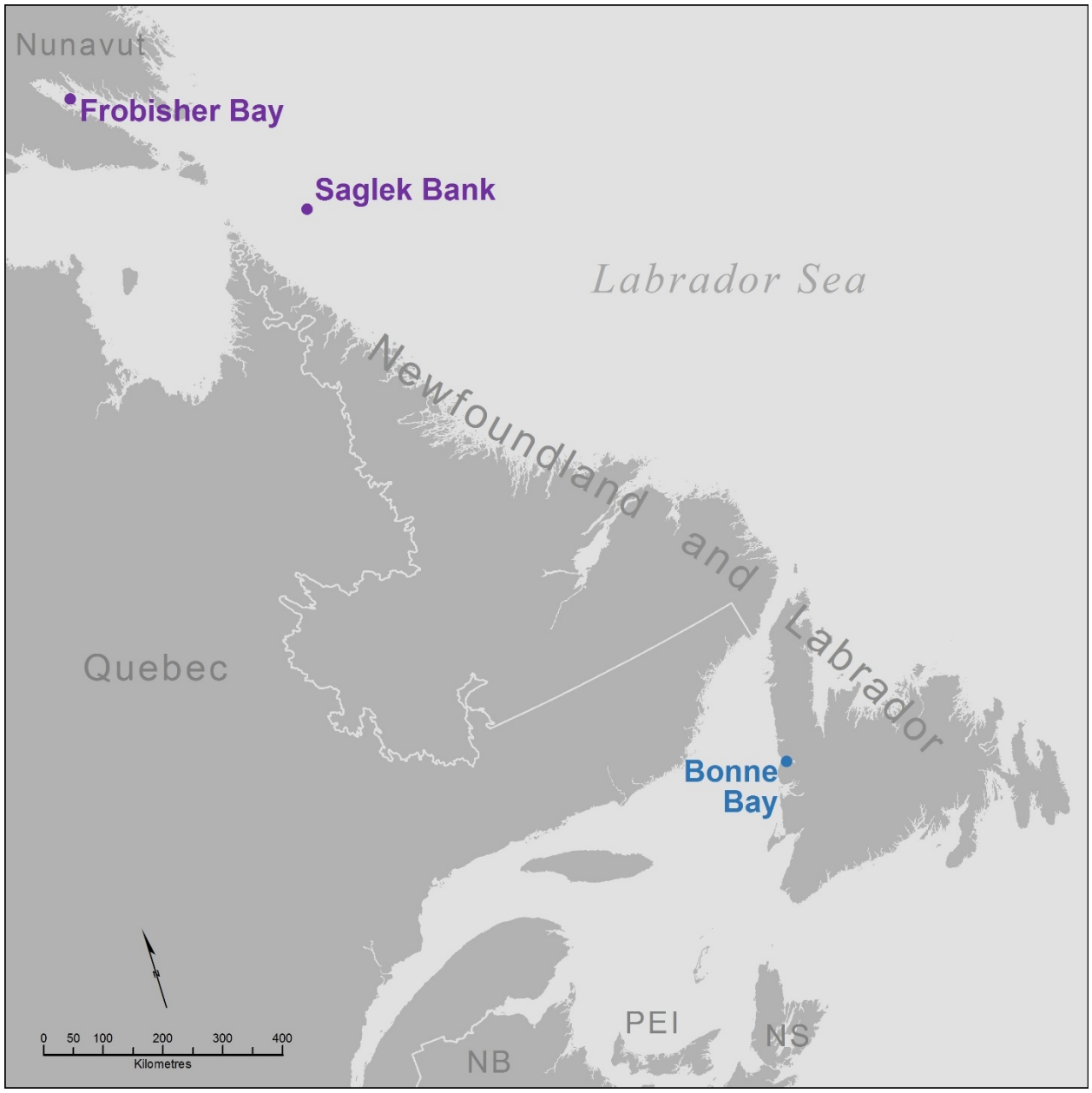

Figure 2.1. Map of the collection locations. Arctic sites in purple: Frobisher Bay (63°17’N, 67°47'W) and Saglek Bank (60°18'N, 62°11′W); subarctic site in blue: Bonne Bay (49°30′N, 57°55′W), Newfoundland. NB: New Brunswick; NS: Nova Scotia; PEI, Prince Edward Island.

notes indicate that five specimens from accession number 414973 were selected as neotypes of *T. gouldi* by K. Ockelmann. In addition, we examined 2 syntypes of *T. plana* from the Smithsonian National Museum of Natural History, accession number USNM 159893.

2.3.2 18S and 28S rRNA gene sequencing and phylogenetic analysis

We sequenced fragments of nuclear 18S (N = 42 from Frobisher Bay, N = 7 from Saglek Bank) and 28S (N = 1 from Frobisher Bay, N = 7 from Saglek Bank) rRNA genes from gills of Arctic thyasirids that were individually kept in 95% ethanol, as in Batstone *et al*. (2014). DNA was isolated using the QIAgen DNeasy Blood & Tissue Kit, following the spin-column protocol for animal tissues. Polymerase chain reaction (PCR) amplifications were performed using gene-specific sets of primers as in Batstone *et al*. (2014): 1) 18S-5' (Winnepenninckx *et al.* 1998) and 18S1100R (Williams *et al.* 2003) for a ~1000 base pair (bp) fragment of the 18S rRNA gene; 2) LSU-5' (Littlewood *et al.* 2000) and LSU1600R (Williams *et al.* 2003) for a ~1500 bp fragment of the 28S rRNA gene. The PCR was performed using 12.5 µl of Green Dream Master Mix, 1.5 µl of template DNA, 1 µl of forward primer, 1 µl of reverse primer, and 9 µl of water (McCuaig *et al.* 2017). Thermocycler conditions were as follows: 4 min of initial denaturation at 94°C, followed by 35 cycles at 94°C for 30 s, 30 s at annealing temperatures of 54°C and 52°C for 18S and 28S, respectively, 2 min at 72°C, with a final elongation at 72°C for 5 min (Batstone *et al.* 2014). Products were cleaned using Agencourt AMPure XP Beads (Beckman Coulter, Brea, CA, USA) following the manufacturer's instructions, and sent to The Center for Advanced Genomics, Toronto, Canada for Sanger sequencing.

For each gene, we manually trimmed sequences at both ends and combined the corresponding forward and reverse sequences from a single specimen into contiguous sequences (contigs) using Sequencher (v. 5.0, Gene Codes Corp. Ann Arbor, MI, USA.). We used Basic Local Alignment Search Tool (BLAST, Altschul *et al.* 1990) to find closely related sequences in GenBank. Contigs were aligned and compared in MEGA 7 (Kumar *et al.* 2016) using the ClustalW algorithm (Thompson *et al.* 1994) and we selected one representative sequence per OTU and gene combination for subsequent phylogenetic analysis. A maximum likelihood tree for concatenated 18S rRNA and 28S rRNA genes was constructed in MEGA 7 using the Kimura 2-parameter model (Nei & Kumar 2000), using our sequences along with other selected thyasirid gene sequences from GenBank. A discrete Gamma distribution was used to model evolutionary rate differences among sites, with some sites allowed to be invariable.

2.3.3 Morphological characterization

We imaged the right and left external valve, internal and external anatomy (where possible), and prodissoconch (the umbonal region) on a Leica MZ95 and an Olympus SZ 61 dissecting microscope. The prodissoconch length (to the nearest 1 μm) and shell dimensions (to the nearest 0.01 mm) were determined using a measuring tool in ImageJ (Abramoff *et al.* 2004). Comparisons of shell dimensions across taxa were performed using one-way ANOVAs in R 3.3.1 (R Core Team 2013), for shell height, length, height/length, tumidity (maximum inflation of a single valve), tumidity/length and prodissoconch length.

Comparisons of shell outlines followed Batstone *et al.* (2014), tracing the outline of the left valve of 37 Bonne Bay specimens (those investigated in Batstone *et al*. 2014) and 13

Thyasira gouldi s.s. individuals [MCZ accession numbers 14394, 14434 (2 specimens), 14494, 14463, 15138 (5 specimens), 192062 (3 specimens)] was traced using the E-Snake module (Delgado-Gonzalo *et al.* 2012) in Image J (Abramoff *et al*. 2004). Shapes were normalized for size and aligned in the same manner (umbo located at the top of each image). We used elliptical Fourier analysis to describe each contour as a series of harmonics and ran a Principal Components Analysis (PCA) of the elliptical Fourier coefficients using Shape 1.6 (Iwata & Ukai 2002). Shape variation along the first two significant principal components (PC 1 and PC 2) was represented on a graph, and reconstructions of shell contours from each extremity of the PC 1 and PC 2 axes were added to represent variation in form. Finally, we ran a cluster analysis of PCA matrix coordinates (first 10 harmonics) based on Euclidian distances in Primer 6.0 (Clarke & Gorley 2006).

2.3.4 Transmission electron microscopy of gills

Gills retrieved for morphological analyses from Frobisher Bay (N = 3) and Saglek Bank (N = 3) were post-fixed in 1% osmium tetroxide in filtered (0.2 µm) seawater for 1 h, dehydrated in an ascending ethanol series and embedded in EPON resin (Electron Microscopy Sciences). Semi-thin (1 µm) sections were made using a LKG Bromma 8800 ultramicrotome, and stained with 1% toluidine blue in 1% sodium borate for light microscopy. We then post-stained ultra-thin sections (60 µm) of each gill were post-stained with Uranyless (Electron Microscopy Sciences) and lead citrate before observing them on a FEI Tecnai Spirit Transmission Electron Microscope.

2.3.5 Stable isotope analyses

We dissected the 17 frozen Saglek Bank bivalves and placed individual tissue samples (all organs combined as dissections of thawed tissues were not feasible) in pre-weighed tin caps (unfolded) and dried them overnight at 70°C. The corresponding valves of each specimen were placed in vials for drying in a muffle oven uncapped at 300°C for 4 hours to burn off organic matter. The dried shells were crushed to a fine powder using a smooth mortar and pestle, then weighed out into sample sizes of 0.2 mg (with a 10% margin). We prepared a duplicate of each shell sample when possible. Shell and tissue samples were weighed and analyzed at the CREAIT Stable Isotope Laboratory at Memorial University using a Finnigan MAT252 Gas Source IRMS coupled with a DeltaVPlus I isotope ratio mass spectrometer interfaced with the Carlo Erba NA1500 Series II Elemental Analyzer. We calibrated isotopic compositions using internal standards and references from Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric air for $\delta^{15}N$, and compared isotope ratios of the Saglek Bank to values obtained previously from Bonne Bay specimens (Zanzerl *et al*. 2019 and unpublished data).

2.4 Results

2.4.1 18S and 28S rRNA sequencing and phylogenetic analysis

We successfully obtained 44 18S rRNA sequences and 8 28S rRNA sequences from the gill tissues of thyasirids from Frobisher Bay and Saglek Bank. Each sequence was identical to one previously obtained from the *Thyasira* cf. *gouldi* complex of three OTUs originally described in Bonne Bay, NL (Batstone *et al.* 2014). We could therefore assign specimens from the Arctic to one of the three known *T*. cf. *gouldi* OTUs; most specimens from Frobisher Bay belonged to OTU 3, while the majority of examined specimens from Saglek Bank consisted of

OTU 1 (Table 2.1). Only one OTU 2 specimen was confirmed among the Arctic thyasirids examined.

The phylogenetic tree for concatenated 18S rRNA and 28S rRNA gene fragments shows that the three OTUs form a distinct cluster, with bootstrap support of 84 (Fig. 2.2). The sister of the *Thyasira* cf. *gouldi* OTUs is a specimen identified as *T. gouldi*, sampled from Port Alberni, British Columbia (accession numbers JF899224 and JF899196, mistakenly stated as having been collected from Mill Bay, UK in Distel *et al*. 2011; P. Dando, pers. comm.). Notably, GenBank has no sequences available for *T. gouldi* specimens collected from (or close to) the Massachusetts type location. Another specimen identified as *T. gouldi* and sampled from Firth of Forth, UK (accession number AJ581871.1) grouped closely with *T. polygona* (Fig. 2.2) rather than with *T. gouldi* or *T*. cf. *gouldi*. Branching support within the *T*. cf. *gouldi* OTU clade was too low (65) to provide strong support for phylogenetic relationships among the three OTUs (Fig. 2.2). A Neighbour-joining tree of COI sequences obtained from Bonne Bay specimens of the three *T*. cf. *gouldi* OTUs, based on data from Batstone *et al.* (2014), provided further evidence of strong support for each OTU (bootstrap values of 99), but low support (bootstrap values <50) for internal branching order (data not shown).

Table 2.1. Number of confirmed specimens from each OTU, per sampling site.

	OTU 1	**OTU 2**	**OTU 3**
Frobisher Bay	1	1	35
Saglek Bank	5	0	2
Bonne Bay	37	5	17

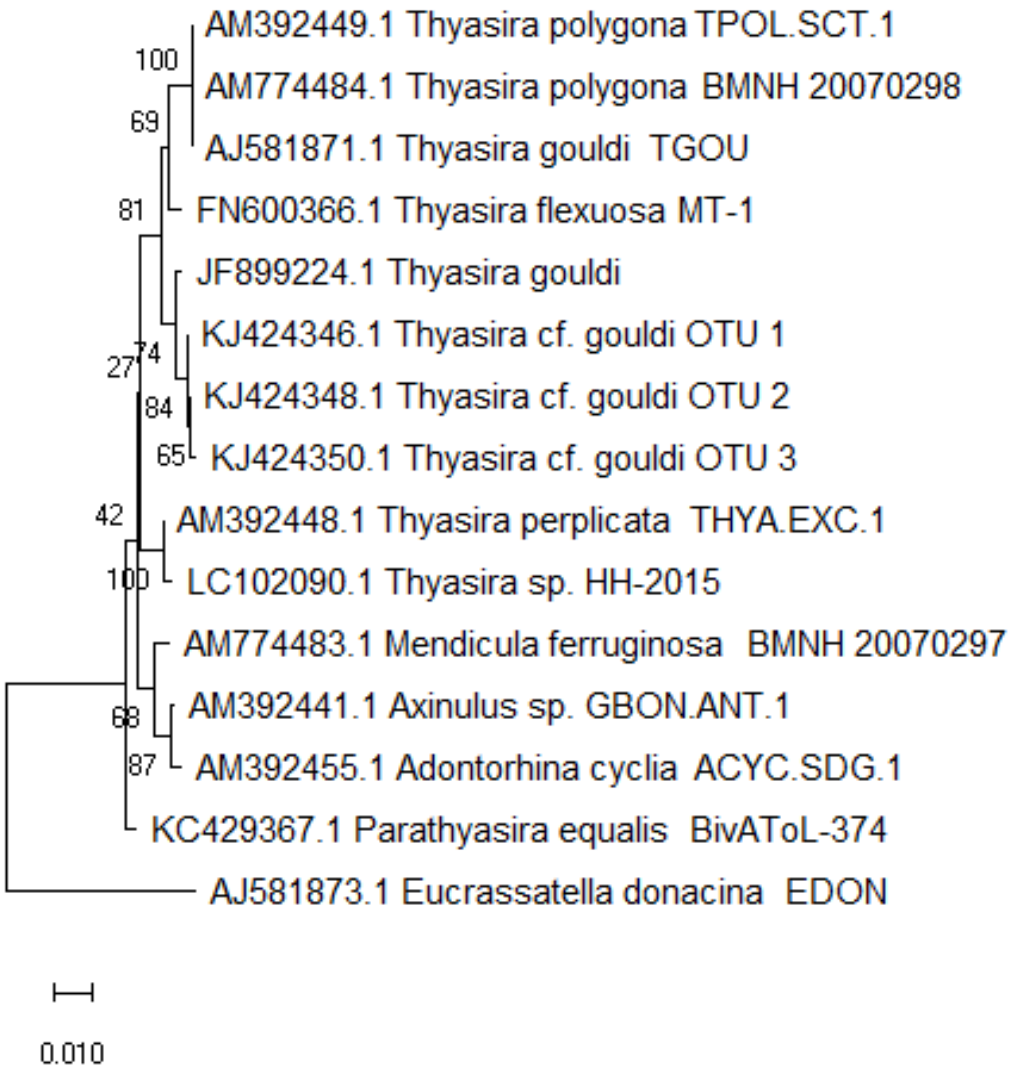

Figure 2.2. Maximum likelihood tree for the concatenated 18S rRNA and 28S rRNA genes, based on the Kimura 2-parameter model. Branch lengths show number of substitutions per site; the percentage of trees in which associated taxa clustered together is shown next to the branches (where over 50%). We used a discrete Gamma distribution to model evolutionary rate differences among sites, allowing some sites to be invariable. The analysis involved 15 nucleotide sequences with 1308 positions in the final dataset. The tree with the highest log likelihood is shown. Analysis used MEGA7.

2.4.2 Symbiont presence in Arctic specimens

TEM observations revealed symbionts in the gill filaments of Arctic OTU 1 specimens (no specimens of OTU 2 were available for TEM examination). The symbionts, present in large numbers, resemble those described in Bonne Bay (Batstone *et al.* 2014). Symbionts were located extracellularly in epithelial cells at the enlarged abfrontal end of gill filaments (Fig. 2.3). There was no ultrastructural evidence of methanotrophy (stacked intracytoplasmic membranes; Trotsenko & Murrell 2008) although the Saglek Bank location was potentially a hydrocarbon seep. OTU 3 specimens lacked symbionts and had a similar gill structure and ultrastructure as described previously (Batstone *et al.* 2014).

2.4.3 Shell characteristics of *Thyasira* cf. *gouldi* OTUs and *T. gouldi* sensu stricto

Measurements of shell dimensions allowed us to compare the symbiotic and asymbiotic *Thyasira* cf. *gouldi* OTUs to each other, as well as to *T. gouldi* s.s. from Massachusetts. For these analyses, we grouped together the symbiotic *T.* cf. *gouldi* OTUs 1 and 2 (hereafter referred to as "1&2") because our examination of shell characters and internal anatomy revealed no apparent difference between these OTUs, as reported previously (Batstone *et al.* 2014). Notably, the two symbiotic OTUs occur in the same sampling locations and share symbiont phylotypes (Batstone *et al*. 2014; Batstone & Dufour 2016). Initial ANOVA runs compared shell dimensions of specimens from three groups (OTUs 1&2, OTU 3, and *T. gouldi* s.s.); for these analyses we combined *T*. cf. *gouldi* specimens from Bonne Bay and from the Arctic to capture characteristics across known geographical locations. Several measurement comparisons yielded significant differences (Tables 2.2 and 2.3) and support our taxonomic hypotheses.

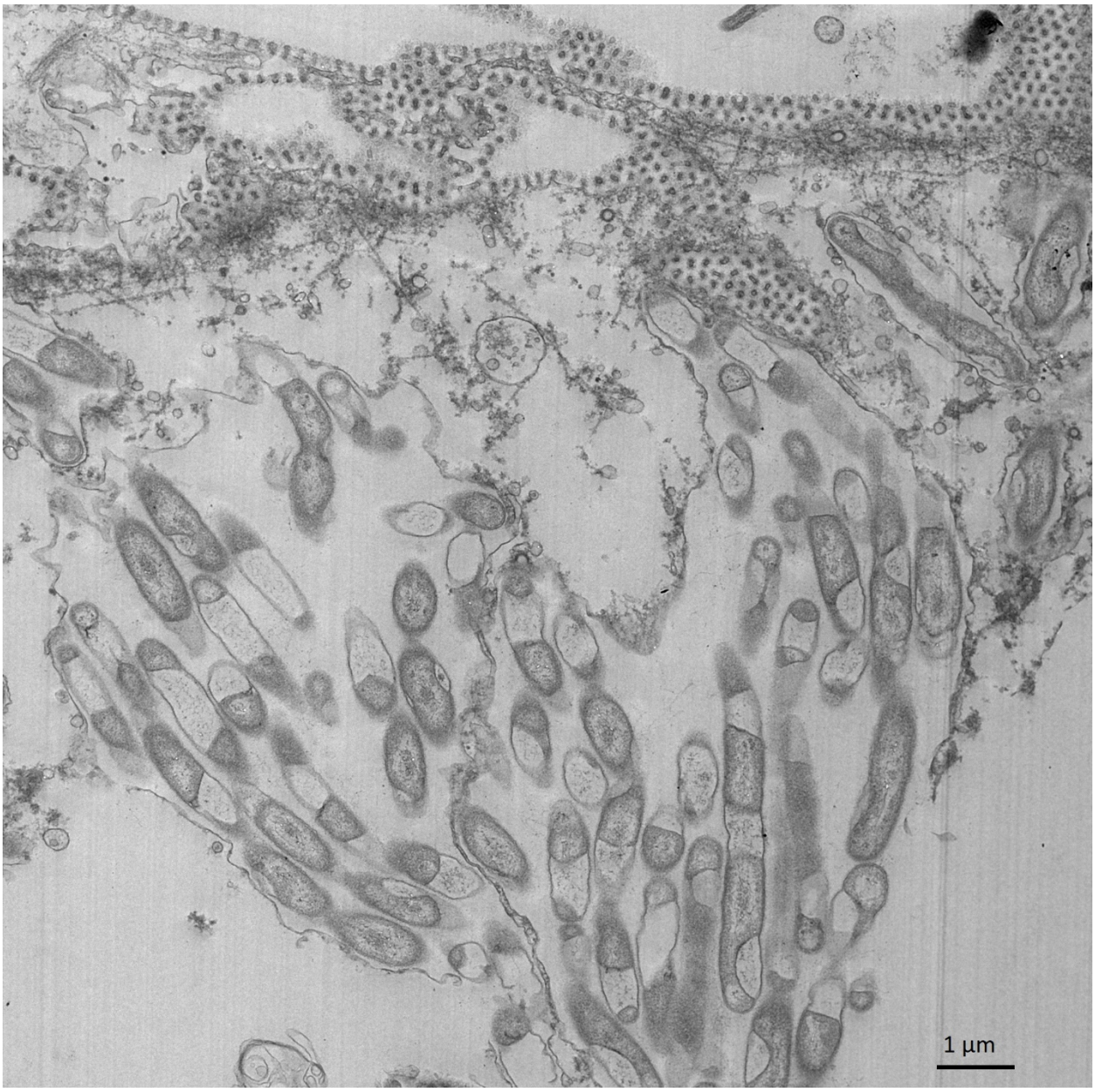

Figure 2.3. Transmission electron micrograph of bacterial symbionts in a thyasirid gill from Saglek Bank.

The shells of asymbiotic *Thyasira* cf. *gouldi* specimens are generally smaller than those of symbiotic OTUs in that the asymbiotic do not reach the maximum size seen in the symbiotic OTUs. The two groups can typically be differentiated on the basis of shell outline and the location of a ferruginous patch at the posterior end of the shell (Batstone *et al*. 2014). Based on the specimens examined herein, shells of OTUs 1&2 are larger and more dorso-ventrally elongate (L/H: 0.99), on average, than those of OTU 3 (L/H: 1.02) (Table 2.3). Shell lengths and heights are significantly smaller in OTU 3 than in OTUs 1&2, but post-hoc analyses of ANOVA results indicate no significant difference in L/H between those groups (Tables 2.2, 2.3). The significantly greater tumidity of OTU 1&2 specimens (mean: 1.13 mm) than that of OTU 3 specimens (mean: 0.89 mm), contrasts no significant difference in tumidity/length values (Table 2.3). Similar prodissoconch size ranges in symbiotic and asymbiotic OTUs parallel no significant difference in prodissoconch size between those two groups (Table 2.3).

Shells of *Thyasira gouldi* specimens from the type location resemble *T.* cf. *gouldi* OTUs, especially OTUs 1&2, in outline and dimensions (Tables 2.2, 2.3; Fig. 2.4). Analyses of shell outline using elliptical Fourier coefficients showed considerable overlap between Bonne Bay and *T. gouldi* s.s. specimens (Figs. 2.5, 2.6). One-way ANOVAs and post-hoc Tukey tests showed significantly smaller shell length, height, and tumidity in *T.* cf. *gouldi* OTU 3 than in both *T.* cf. *gouldi* OTUs 1&2 and *T. gouldi* s.s., but no significant differences between the latter two groups (Tables 2.2 and 2.3). However, the prodissoconchs of *T. gouldi* s.s. were significantly larger (mean: 211 µm) than those of both *T*. cf. *gouldi* OTUs 1&2 (mean: 193 µm) and OTU 3 (mean: 190 µm). The taxonomic importance of prodissoconch size in thyasirids and its positive relationship with increasing latitude (Ockelmann, pers. comm., in Oliver & Killeen 2002)

suggest low likelihood that the *T*. cf. *gouldi* OTUs are conspecific with *T. gouldi* s.s. given the significantly larger prodissoconch sizes at the lowest latitude (Tables 2.2, 2.3).

Based on the morphological and ecological (symbiosis-related) evidence, we infer that the cluster of *Thyasira* cf. *gouldi* OTUs from Bonne Bay, Frobisher Bay and Saglek Bank encompasses two taxa: 1) *T.* cf. *gouldi*., a symbiotic taxon that includes OTUs 1&2, which are possible subspecies or incipient species; and 2) *T. plana*, an asymbiotic species consisting of OTU 3, that was previously synonymized with *T. gouldi* (Ockelmann 1961). While OTUs 1 and 2 could be interpreted as separate species on the basis of molecular differences, we consider them as a single species for reasons of a practical nature (i.e., our investigation revealed no morphological characters that allowed the separation of OTUs 1 and 2). With the genetic data currently available, we cannot confidently resolve branching or sister-group relationships within the *T*. cf. *gouldi* clade. We also lack sufficient data to compare *T*. cf. *gouldi* OTUs 1 and 2 to *T. gouldi* s.s. properly, particularly gene sequences from the type location, and therefore retain a more tentative designation for these bivalves.

Table 2.2. Results of one-way ANOVA tests comparing shell dimensions of symbiotic *T*. cf. *gouldi* (OTUs 1 & 2 combined), asymbiotic *T*. cf. *gouldi* (OTU 3) and *Thyasira gouldi* s.s. from Massachusetts. For *T*. cf. *gouldi*, specimens from Bonne Bay, Frobisher Bay and Saglek Bank were combined.

	df	F	p
Height	2, 84	15.1803	< 0.0001
Length	2, 83	11.9741	< 0.0001
Length/Height	2, 84	0.0093	0.9907
Tumidity	2, 82	10.3149	< 0.0001
Tumidity/Length	2, 81	4.3523	0.0160
Prodissoconch	2, 104	4.5936	0.0112

Table 2.3. Shell dimensions of symbiotic *T*. cf. *gouldi* (OTUs 1&2), asymbiotic *T*. cf. *gouldi* (OTU 3) and *Thyasira gouldi* s.s. from Massachusetts. For *T*. cf. *gouldi*, specimens from Bonne Bay, Frobisher Bay and Saglek Bank were combined.

	Mean	Std. dev.	Count	Min	Max
***T.* cf. *gouldi* OTUs 1&2**					
Length (mm)	3.66[a]	0.69	51	2.36	5.04
Height (mm)	3.70[a]	0.73	51	2.36	5.19
Length/Height	0.99	0.95	51	1.00	0.97
Tumidity (mm)	1.13[a]	0.24	50	0.64	1.62
Tumidity/Length	0.31[a]	0.02	50	0.26	0.36
Prodissoconch (µm)	193[a]	21	49	155	238
***T. cf. gouldi* OTU 3**					
Length (mm)	2.95[b]	0.47	25	2.33	3.94
Height (mm)	2.88[b]	0.49	26	2.20	3.99
Length/Height	1.02	0.96	26	1.06	0.99
Tumidity (mm)	0.89[b]	0.18	25	0.69	1.40
Tumidity/Length	0.30[a,b]	0.02	24	0.24	0.36
Prodissoconch (µm)	190[a]	23	46	130	219
***T. gouldi* s.s.**					
Length (mm)	3.79[a]	0.67	10	2.78	4.88
Height (mm)	3.84[a]	0.66	10	2.81	4.88
Length/Height	0.99	1.01	10	0.99	1.00
Tumidity (mm)	1.10[a]	0.19	10	0.78	1.37
Tumidity/Length	0.29 [b]	0.02	10	0.27	0.33
Prodissoconch (µm)	211[b]	7	12	200	220

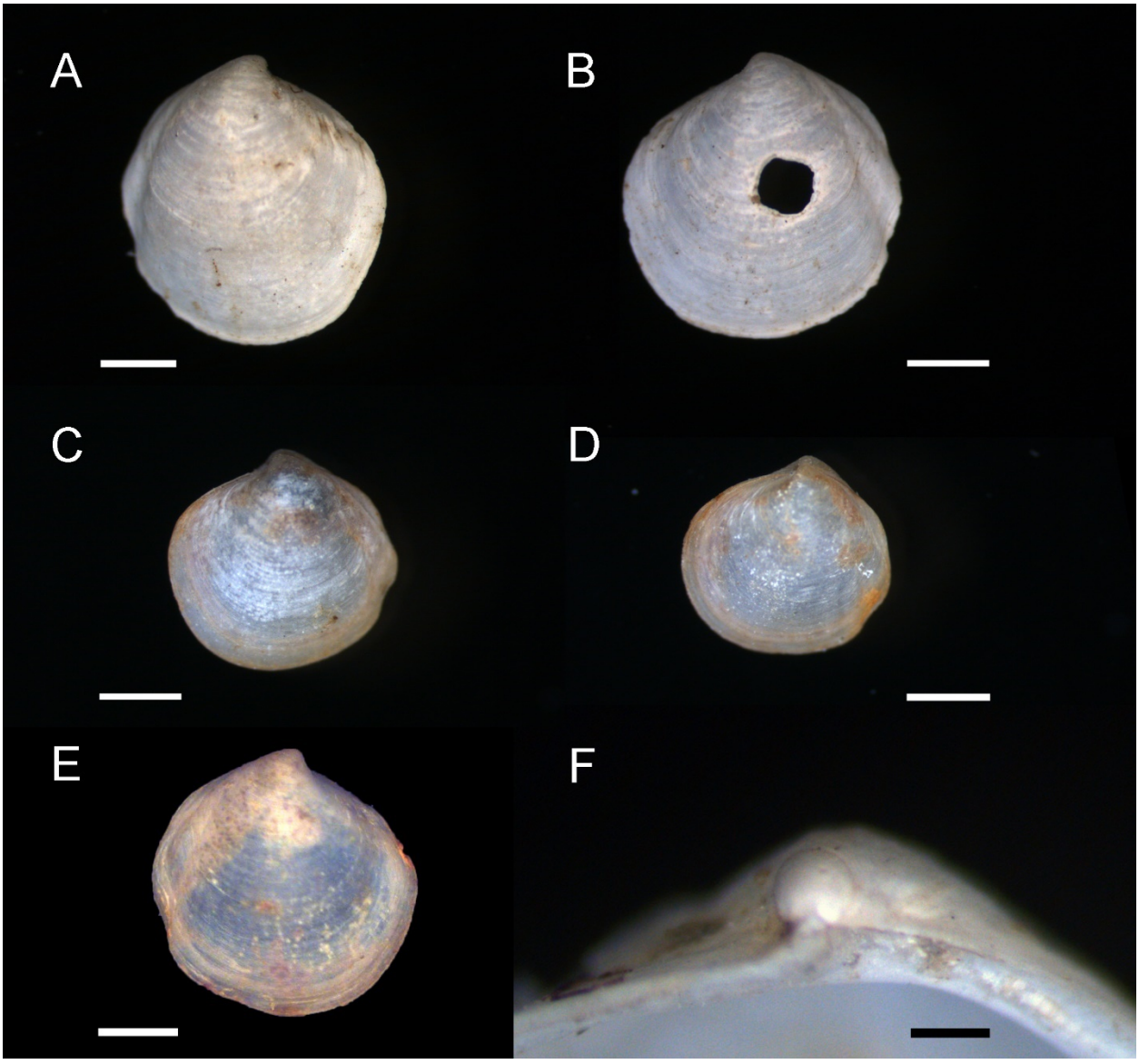

Figure 2.4. ***Thyasira gouldi*** **sensu stricto,** from type location (Massachusetts). All shells are from the Museum of Comparative Zoology, Harvard University Accession Number 414973, catalog number 15138. (A–E) View of a single valve. Scale bars = 1 mm (F) View of prodissoconch. Scale bar = 200 µm.

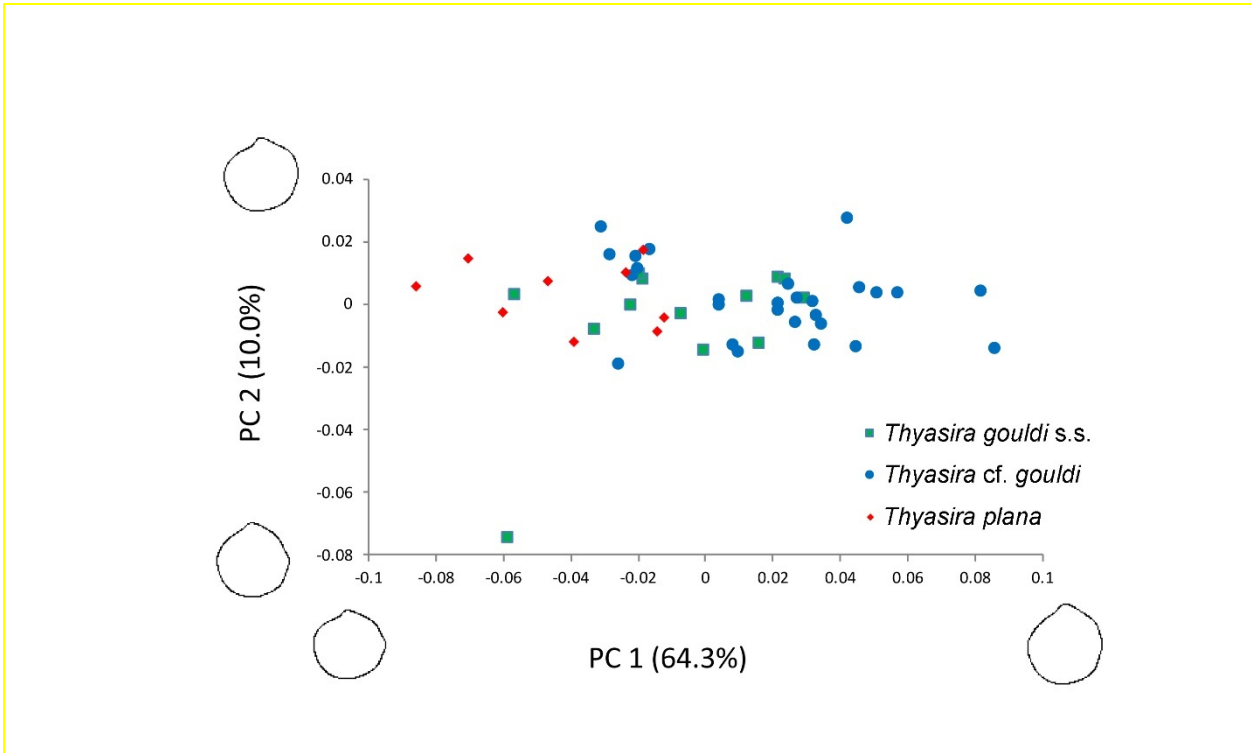

Figure 2.5. Variation in shell shape along the first two principal components. Shapes represented at the end of each axis were reconstructed using Shape 1.6.

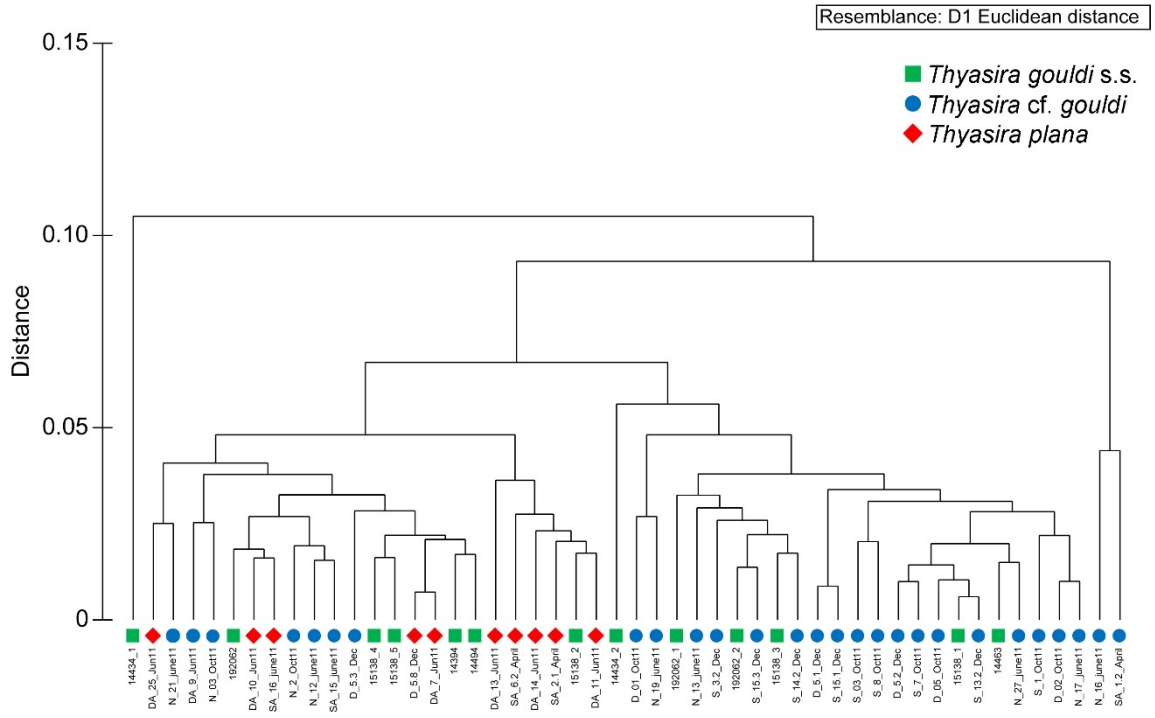

Figure 2.6. Cluster analysis of shell outlines based on Elliptical Fourier Analysis, considering the first 10 harmonics. Shells do not cluster according to species identity.

2.4.4 Descriptions

The terminology for shell structures and morphology follows that of Oliver & Killeen (2002).

Anatomical abbreviations. aa, anterior adductor muscle; agr, anterior glandular ridge; ct, ctenidium; dg, lateral body pouch; f, foot; id, inner demibranch; lbp, lateral body pouch; lp, labial palps; od, outer demibranch; pa, posterior adductor muscle; pgr, posterior glandular ridge; ppr, posterior pedal retractor muscle; r, rectum.

Institutional abbreviations. CMN, Canadian Museum of Nature; NFM, The Rooms Corporation of Newfoundland and Labrador; MCZ, Museum of Comparative Zoology; USNM, Smithsonian National Museum of Natural History.

2.4.5 Taxonomy

Thyasira Leach in Lamarck, 1818

Type species. *Tellina flexuosa* Montagu, 1803

Diagnosis. Fragile shells, subcircular, ovate to ovate-polygonal in outline with a posterior sulcus; escutcheon variably expressed, absent to deep, submarginal sulcus with or without an auricle. Hinge teeth lacking or as a single “cardinal” tubercle, ligament sunken. Anterior adductor scar elongate, posterior adductor scar ovate, pallial line entire. Ctenidium with two demibranchs, lateral body pouches large and multilobed, foot vermiform, heel obsolete, toe developed.

Thyasira* cf. *gouldi

Material examined. Six live-collected specimens, Southeast Arm, Bonne Bay, NL (49°27'N, 57°43'W), 30 m depth, October 2016, collector S. Dufour, preserved in 70% ethanol, four of which were deposited at the CMN, accession numbers CMNML 2023-0528 (N = 1), CMNML 2023-0529 (N = 3) and two of which were deposited at The Rooms Corporation of Newfoundland and Labrador, accession numbers NFM MO-2889; and 3 live-collected specimens from Saglek Bank (60°18'N, 62°11'W), 275 m depth, July 20, 2016, collector R. Dove, preserved in 70% ethanol, deposited at the CMN, accession numbers CMNML 2023-0530, CMNML 2023-0531, CMNML 2023-0532.

Description. Shell (Fig. 2.7): Up to 5 mm in length (summary statistics of shell dimensions are presented in Tables 2.3, as OTUs 1&2, and 2.4); thin shelled; equivalve; subequilateral, beaks a little to the anterior. Relatively tumid (mean tumidity/length: 0.31). Outline typically subequilateral to roundly-subovate; height a little greater than or equal to length, some specimens with length slightly greater than height; umbos projecting, beaks slightly prosogyre; anterior expanded laterally. Lunule margin long, sloping, large, and slightly sunken; junction of lunule margin and anterior margin rounded to subacute; anterior – ventral margin narrowly rounded and angulated laterally, forming a continuous rounded curve with the ventral margin; posterior margin bisinuate with posterior sinus being more distinct than the marginal sinus and more defined in larger shells (Figs. 2.7A, C–H); auricle low and almost extending the length of the submarginal sulcus; ligament sunken, visible for about half the length of the auricle (Fig. 2.7B), and set on a shallow resilium. Posterior area has two distinctly rounded folds giving rise to the submarginal sulcus and a sharp posterior sulcus (Fig. 2.7A). Hinge has a weakly defined small

tooth in the right valve, sometimes difficult to locate due to it being eroded in some specimens; left valve has a corresponding small depression below the beak. Sculpture of regular concentric lines and growth stops with weakly apparent dents, damage marks, and ridges. Periostracum thin, silky, and translucent over white shell; small ferruginous patches sometimes visible near the posterior sinus (Fig. 2.7A), and the anterior – lunule margin junction (Figs. 2.7C–E). Prodissoconch (Fig. 2.7B) medium, 155–238 μm in length, mean = 193 m. The larval shell increases in size as the latitude increases (Table 2.4).

Anatomy (Figs. 2.8A, B): Mantle edge fused posteriorly with gill axis and posterior adductor to form an exhalent aperture; mantle thin, transparent; ventral and anterior margins not fused; glandular tissue on inner side thin (Fig. 2.8A). Foot creates an inhalant aperture below the anterior adductor; vermiform with a bulbous tip (Fig. 2.8B). Anterior adductor larger and elongate, whereas the posterior adductor is short and oval (Fig. 2.8A). Labial palps small and poorly developed, sorting ridges are indistinct (Fig. 2.8A). Ctenidium consist of both inner and outer demibranchs, both as thick fleshy lamellae, outer demibranch is about half the length of the inner (Fig. 2.8B). Lateral body pouch distinct and greatly divided, extensively lobed and branched (Fig. 2.8B). Rectum easily visible as a pale thin tube above the posterior pedal retractor (Fig. 2.8A).

Remarks. The *Thyasira* cf. *gouldi* material studied here cannot currently be confirmed as *T. gouldi* s.s., given that our analysis is restricted to shell features. Although we observed no clear difference in outline between *T.* cf. *gouldi* and *T. gouldi* s.s. shells, we noted that prodissoconch sizes did not correspond to the latitudinal pattern expected (see Discussion, below). Until we are

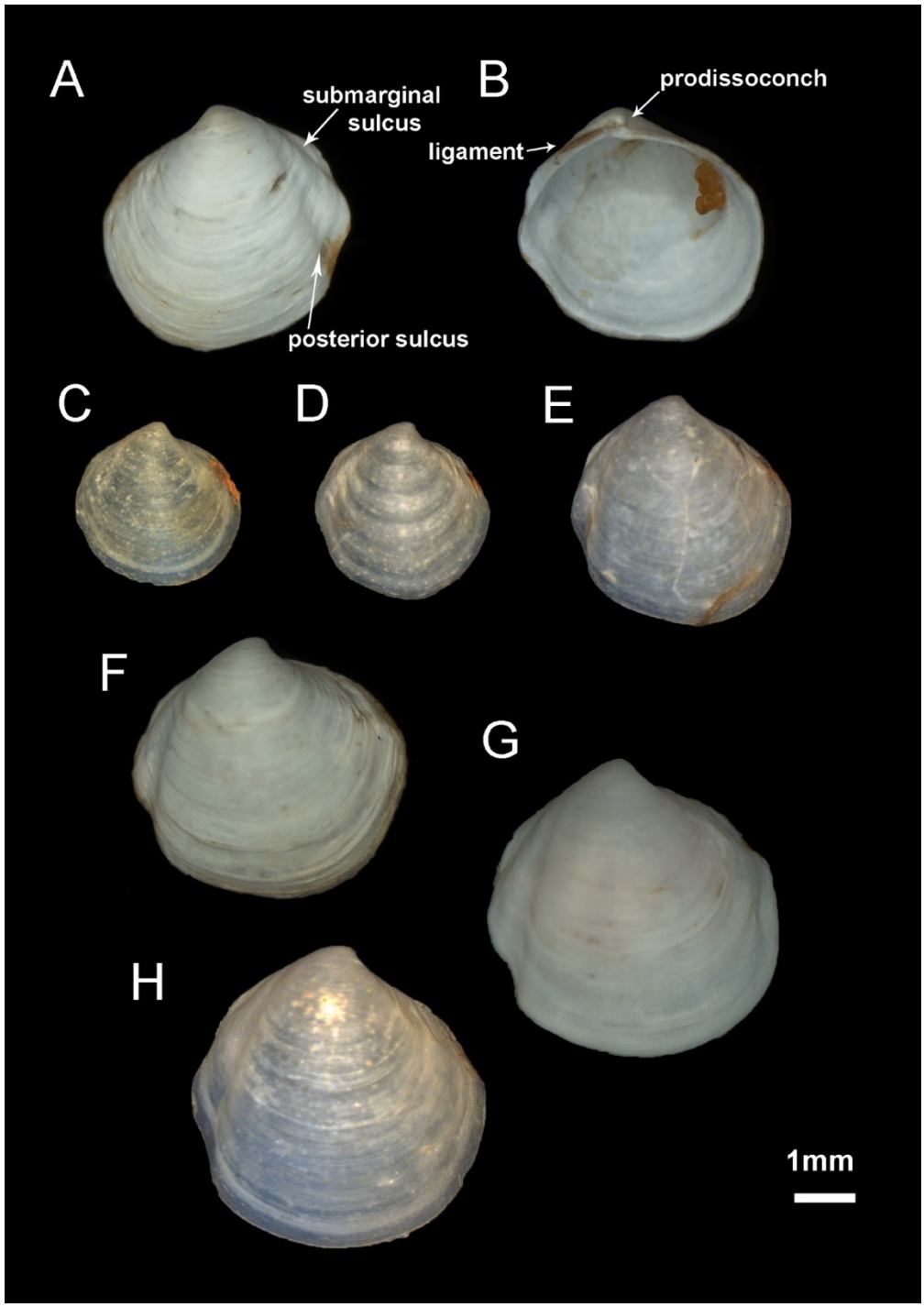

Figure 2.7. ***Thyasira*** **cf.** ***gouldi.*** (A) external and (B) internal view of left valve; (C–E) shells of juveniles; (F–G) large shells.

able to obtain gene sequences from the type location, we retain the designation of *T.* cf. *gouldi* for specimens from Bonne Bay and the eastern Canadian Arctic.

Table 2.4. Descriptive statistics of shell dimensions for *Thyasira* cf. *gouldi* comparing subarctic (Bonne Bay) and Arctic sites (Frobisher Bay and Saglek Bank). Tumidity is measured from a single valve.

	Mean	**Std. Dev.**	**Count**	**Min**	**Max**
Subarctic					
Length (mm)	3.52	0.64	42	2.36	5.04
Height (mm)	3.58	0.70	42	2.36	5.19
Tumidity (mm)	1.10	0.24	42	0.64	1.62
Tumidity/Length	0.31	0.02	42	0.26	0.36
Prodissoconch (µm)	184	17	35	155	215
Arctic					
Length (mm)	4.29	0.54	9	3.28	4.93
Height (mm)	4.28	0.58	9	3.26	5.06
Tumidity (mm)	1.29	0.21	8	0.94	1.54
Tumidity/Length	0.30	0.02	8	0.28	0.33
Prodissoconch (µm)	215	12	14	202	238

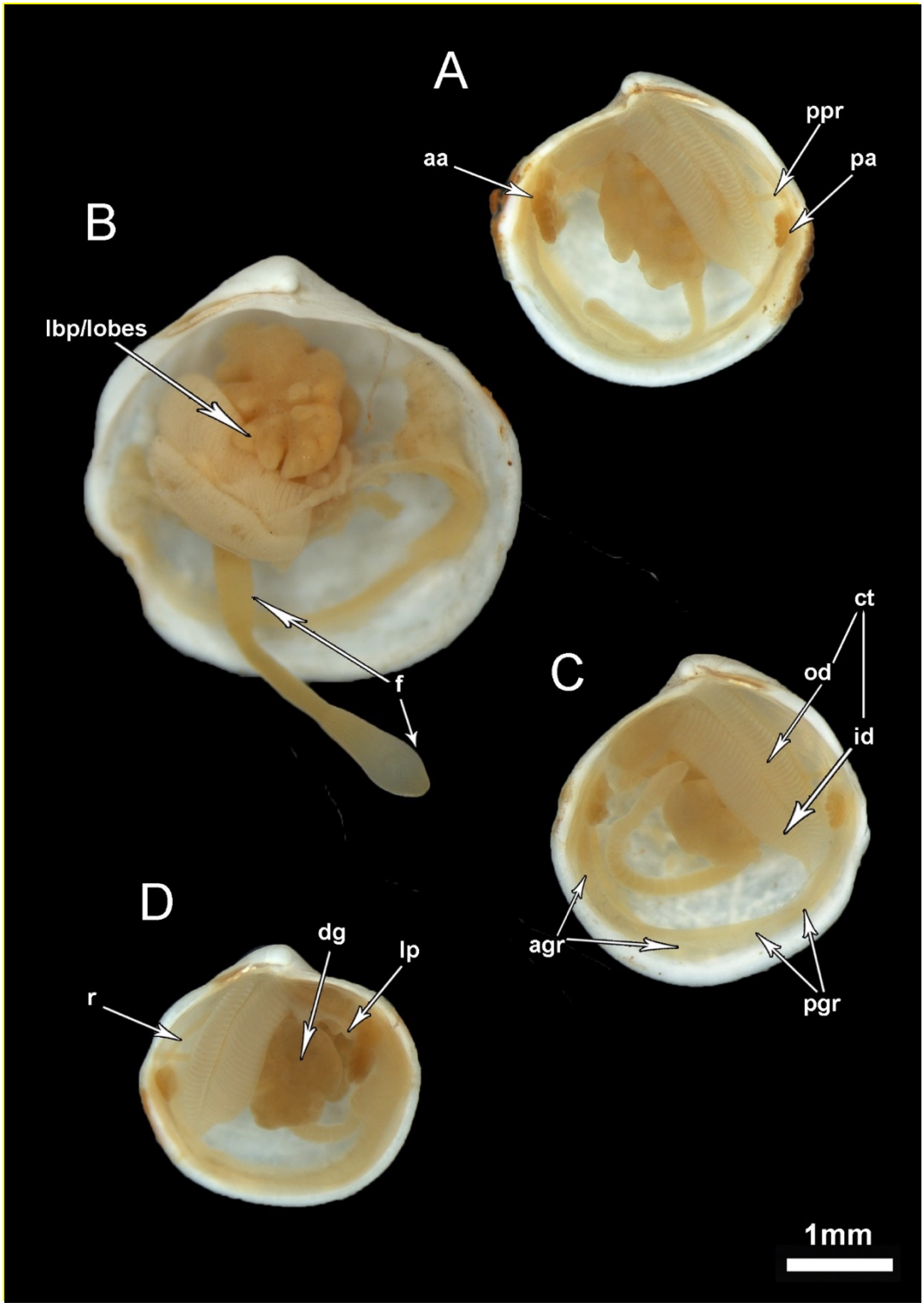

Figure 2.8. ***Thyasira*** **cf.** ***gouldi.*** (A) Gross anatomy; (B) Gross anatomy, with ctenidium detached and orientated to show lobed lateral body pouch. (C, D) *Thyasira plana* gross anatomy.

***Thyasira plana* (Verrill & Bush, 1898)**

Type locality: North of Cape Cod, in the Gulf of Maine, Casco Bay, Bay of Fundy, and Halifax Harbor. Depth: 14–183 m. *Syntype locality*: USFC St. 254, Cape Cod Bay, off Wood End Light 41.95 N, 70.2917 W. Depth: 44 m.

Material examined. Seven live-collected specimens, Southeast Arm, Bonne Bay, NL (49°27'N, 57°43'W), 30 depth, July 2017, collector S. Dufour, preserved in 70% ethanol, of which one was deposited at the CMN, accession number CMNML 2023-0533 and 6 others were deposited at The Rooms Corporation of Newfoundland and Labrador, accession numbers NFM MO-2890 and NFM MO-2891; 2 live-collected from Frobisher Bay, 62°58'N, 67°16'W, 572 m depth, July 17, 2016, collector R. Dove, preserved in 70% ethanol, accession numbers CMNML 2023-0534, CMNML 2023-0535; 1 from Frobisher Bay, 63°40'N, 68°25'W, 62 m depth, July 16, 2016, collector R. Dove, preserved in 70% ethanol, deposited at the CMN, accession number CMNML 2023-0536; and 1 from Saglek Bank (60°18'N, 62°11'W), 275 m depth, July 20, 2016, collector R. Dove, preserved in 70% ethanol, deposited at the CMN, accession number CMNML 2023-0537.

Description.

Shell (Fig. 2.9A–J): Up to 3.9 mm in length; thin shelled; equivalve; subequilateral, beaks towards the anterior. Relatively tumid (mean tumidity/length: 0.30). Outline typically subequilateral to subovate; length greater than or equal to height, few specimens with height slightly greater than length; umbos projecting, beaks prosogyre; anterior expanded a little

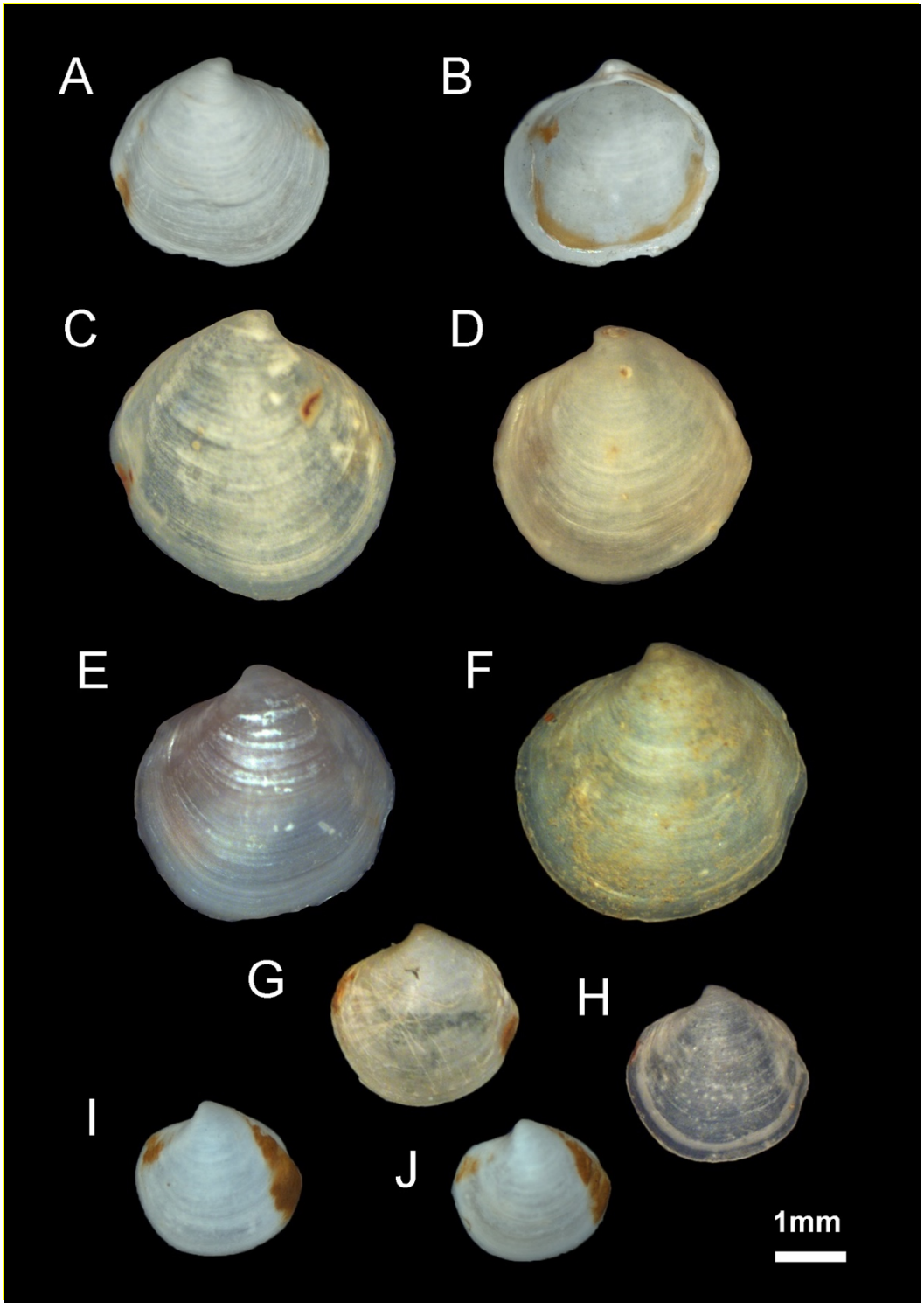

Figure 2.9. ***Thyasira plana.*** (A) external and (B) internal view of right valve; (C–F) large shells; (G–J) juvenile shells.

laterally. Lunule margin moderately long, curved, slightly sloping and not sunken; junction of lunule margin and anterior margin rounded; anterior – ventral margin forming a continuous rounded curve and angulated slightly laterally in larger shells, but not in juvenile shells; posterior margin weakly bisinuate with some juvenile shells only showing the marginal sinus (Figs. 2.9I, J); auricle very low and sometimes absent (Fig. 2.9C); ligament sunken, short and set on a shallow resilium. Posterior sulcus shallow, absent in some specimens (Figs. 2.9D, I, J), submarginal sulcus shallow. Hinge has a weakly defined small tooth in the right valve, often hard to locate; left valve has a corresponding small depression below the beak. Sculpture of regular concentric lines and growth stops with irregular dents, weak ridges, and damage marks. Periostracum thin, translucent, and giving a silky appearance over the white shell; ferruginous patches often visible near posterior sinus (Figs. 2.9C, G), sometimes expanded laterally along the posterior area (Figs. 2.9I, J), and anterior – lunule margin junction (Figs. 2.9A, G, I, J). Prodissoconch small-medium, 130–219 µm in length, mean = 190 µm. The larval shell increases in size as the latitude increases (Table 2.5).

Shell measurements of syntypes: Length 3.64 mm, height 3.62 mm (left valve of syntype from Fig. 2.10); Length 3.65 mm, height 3.64 mm (right valve of syntype in Fig. 2.10).

Anatomy (Figs. 2.8C, D): Mantle thin, transparent; mantle edge fused posteriorly with posterior adductor and where gill terminates to form an exhalent aperture; fusion absent in ventral and anterior margins; glandular tissue on inner side moderately thick, defined anterior glandular ridge (Fig. 2.8C). Foot creates an inhalant aperture below the anterior adductor; elongate, vermiform with a bulbous toe (Fig. 2.8C). Anterior adductor elongated and large, posterior adductor short and oval (Fig. 2.8D). Labial palps small, but developed (Fig. 2.8D), sorting ridges

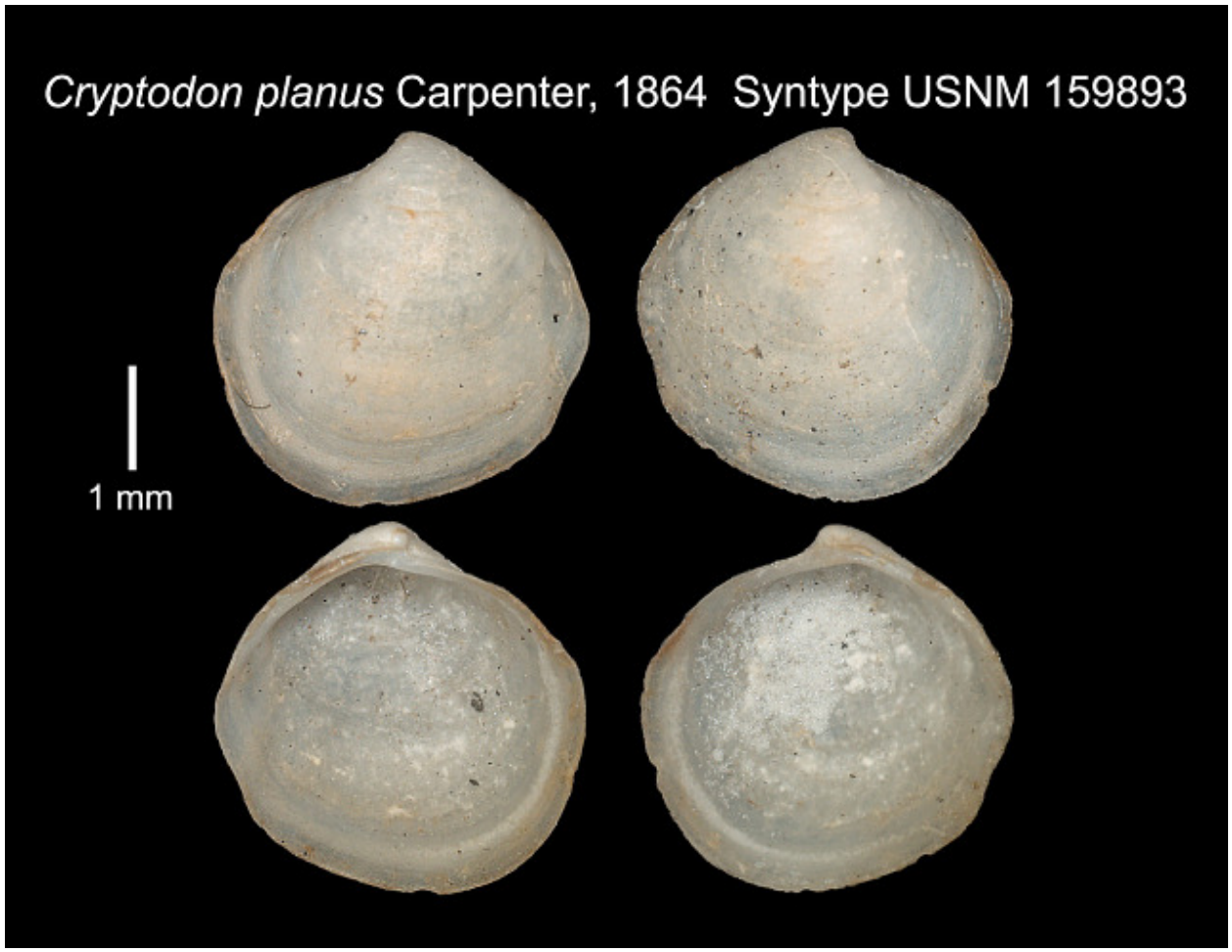

Figure 2.10. ***Thyasira plana.*** Syntypes, as *Cryptodon planus* Carpenter, 1864, from USNM, catalog number USNM 159893. Collected on July 28, 1879. Image from Smithsonian Open Access (CC0).

indistinct. Ctenidium composed of both inner and outer demibranchs, outer demibranch is about half the size of the inner (Fig. 2.8C). Lateral body pouch small, with few lobes; some specimens not showing any lobes, being rather slightly branched (Fig. 2.8D). Rectum easy to locate, pale thin tube above the posterior pedal retractor (Fig. 2.8D).

Remarks. Specimens examined correspond well to the description and figure of *Thyasira plana* in Verrill & Bush (1898) and to the photographs of the syntypes USNM 159893, particularly with regards to the less defined indentation and margin angulation in the posterior shell and the rounded anterior and ventral margins. Specimens genetically distinct from *T*. cf. *gouldi* (although closely related), and the absence of symbionts, which was consistently noted in all specimens examined (Batstone *et al*. 2014) represents a notable difference between those taxa.

2.4.6 Latitudinal patterns in prodissoconch size

The prodissoconch in both *Thyasira* cf. *gouldi* and *T. plana* increases in size with increasing latitude, consistent with latitudinal trends proposed previously by Ockelmann (pers. comm, in Oliver & Killeen 2002). *T.* cf. *gouldi* prodissoconch size ranges between 155 to 215 µm in Bonne Bay while in the Arctic sites (Saglek Bank and Frobisher Bay), it ranges between 202 to 238 µm (Table 2.4). *T. plana* prodissoconch size ranges from 130 to 195 µm in Bonne Bay and from 175 to 219 µm at Arctic sites (Table 2.5). Both taxa differed significantly in size [one-tailed t-tests: *T.* cf. *gouldi*, $t(47) = -6.1817$, $p < 0.0001$; *T. plana*: $t(44) = -8.3264$, $p < 0.001$].

Table 2.5. Descriptive statistics of shell dimensions for *Thyasira plana* comparing sub-arctic (Bonne Bay, NL) and Arctic sites (Frobisher Bay and Saglek Bank). Tumidity is measured from a single valve.

	Mean	Std. Dev.	Count	Min	Max
Subarctic					
Length (mm)	2.75	0.26	16	2.33	3.36
Height (mm)	2.70	0.29	17	2.20	3.33
Tumidity (mm)	0.81	0.08	17	0.69	0.97
Length/Tumidity	0.29	0.01	16	0.28	0.32
Prodissoconch (µm)	163	19	14	130	195
Arctic					
Length (mm)	3.31	0.57	9	2.36	3.94
Height (mm)	3.23	0.62	9	2.21	3.99
Tumidity (mm)	1.05	0.22	8	0.79	1.40
Length/Tumidity	0.30	0.04	8	0.24	0.36
Prodissoconch (µm)	202	12	32	175	219

2.4.7 Stable isotope analysis

We observed the same trend in tissue stable isotope ratios as in Zanzerl *et al*. (2019), with a lower nitrogen isotope ratio in the symbiotic *Thyasira* cf. *gouldi* than the asymbiotic species within each site (Table 2.6). For each species, nitrogen isotope ratios are lower in Bonne Bay than in Saglek Bank. There is little difference in carbon isotope ratios between species and sites.

Within each site, shell carbonate isotopic composition varied more for *Thyasira plana* than for *T.* cf. *gouldi* (Table 2.7). The isotopic composition of *T. plana* shell carbonates spanned a greater range of values among Saglek Bank specimens (difference of 4‰) compared to the Bonne Bay specimens (difference of 2.4‰). The shell carbonates of *T.* cf. *gouldi* showed a relatively low variation in carbon isotopic composition among specimens, and values were slightly more negative at Bonne Bay (Table 2.7).

Table 2.6. Isotopic composition of tissues from *Thyasira* cf. *gouldi* and *Thyasira plana* from Saglek Bank and Bonne Bay. Ratios represent ranges. Bonne Bay data are for non-gill tissues (pooled individuals); see Zanzerl *et al*. 2019.

	Thyasira* cf. *gouldi		***Thyasira plana***	
	Isotope ratio (‰)	**N**	**Isotope ratio (‰)**	
Saglek Bank				
$\delta^{13}C$	-24.55 to -18.87	8	-22.35 to -19.57	4
$\delta^{15}N$	6.52 to 10.1	8	10 to 10.36	4
Bonne Bay				
$\delta^{13}C$	-21.54 to -18.86	16	-21.24 to -19.30	10
$\delta^{15}N$	-0.10 to 5.16	16	6.62 to 8.25	10

Table 2.7. Isotopic carbon composition of shells from *Thyasira* cf. *gouldi* and *Thyasira plana* from Saglek Bank and Bonne Bay. Ratios represent ranges.

	Thyasira* cf. *gouldi		***Thyasira plana***	
	Shell $\delta^{13}C$ (‰)	**N**	**Shell $\delta^{13}C$ (‰)**	**N**
Saglek Bank	-5.3 to -4.5	7	-7.9 to -3.9	5
Bonne Bay	-6.3 to -4.9	5	-7.1 to -4.7	5

2.5 Discussion

2.5.1 Comparisons of Bonne Bay and Arctic specimens to *Thyasira gouldi* sensu stricto

The lack of available soft tissues or genetic data for *Thyasira gouldi* from the type location limited comparison of the Bonne Bay and Arctic thyasirids to *T. gouldi* s.s. to shell features. An examination of the shell of multiple presumed conspecifics from the type location revealed no obvious, consistent differences in outline and curvature between *T. gouldi* s.s. and the Bonne Bay and Arctic specimens examined herein. The analysis of shell outline showed that the *T. gouldi* s.s. shells fell within the broader morphospace occupied by both *T.* cf. *gouldi* and *T. plana* (Fig. 2.5). Intraspecific variation in shell outline is considered typical in thyasirids (Taylor *et al.* 2007), and previous studies report ontogenetic changes in shape (Ockelmann 1958; Kauffman 1969; Killeen & Oliver 2002b; Oliver & Sellanes 2005; Oliver & Drewery 2014), including in *T.* cf. *gouldi* OTUs 1&2 (Batstone *et al*. 2014). Comparisons of shell dimensions indicated that, while *T. plana* was generally smaller than *T. cf. gouldi* and *T. gouldi* s.s., the latter two are similar in length, height, L/H and tumidity, although T/L was significantly greater in *T.* cf. *gouldi* than in *T. gouldi* s.s. (Table 2.3).

Of the shell features examined, prodissoconch size best differentiates *Thyasira gouldi* s.s. and the more northern specimens. Based on ANOVA results, prodissoconch size varied significantly among the three taxa, and the post-hoc Tukey test revealed significant differences between *T. gouldi* s.s. and both *T.* cf. *gouldi* and *T. plana*, but not between the latter two groups. Whereas prodissoconch size within a species could vary across sites, the pattern observed does not conform to expectations if specimens from Bonne Bay and Arctic sites both belonged to the species *T. gouldi* s.s., and therefore suggests separate species. Ascertaining whether *T.* cf. *gouldi* and *T. gouldi* s.s. are conspecific will require investigation of additional features, including gene sequences from *T. gouldi* from the type location. Within *T.* cf. *gouldi* and *T. plana* (two genetically defined taxa), we observed significantly greater prodissoconch size at the higher latitude site. Multiple studies report increases in prodissoconch size with increasing latitude in many molluscs (Thorson 1950, Jablonski & Lutz 1980), including species of thyasirids (Ocklemann 1958). This increase in size likely corresponds to a difference in egg size (Moran 2004) and may be associated with site-specific differences in temperature (as reported in *Tiostrea chilensis*; Jeffs *et al*. 1997). Although we lack specific information on bottom temperatures associated with the *T. gouldi* s.s. specimens examined herein, more recent measurements of near-bottom temperatures from the same sites reported ranges of approximately 2 to 12°C for Cape Cod Bay (Costa *et al*. 2017), and approximately 3 to 15°C for Georges Bank between 1982 and 2015 (Kavanaugh *et al*. 2017). Considering that bottom water temperatures in the region have increased (Kavanaugh *et al*. 2017), the ambient temperatures at the time of sampling for the *T. gouldi* s.s. specimens examined herein (between 1910 and 1945), could have been up to a few °C cooler. Nevertheless, these temperatures approximate those measured within sediments at the Bonne Bay site (yearly range between 2011–2013: 0.7 to 14˚C; unpublished

data), and therefore could not explain the difference in prodissoconch size between these two sites, assuming specimens belong to the same species. In contrast, bottom temperatures of -1.3˚C were measured at the time of sampling in Frobisher Bay, and long-term data from 40 m depth at a nearby site show temperatures ranging from -1.7 to 0.4 ˚C (Lovrity 1981, 1982a, b, 1984, 1987). Temperature differences between Bonne Bay and the Arctic sites could explain the significant, site-specific differences in prodissoconch size in *T.* cf. *gouldi* and *T. plana*.

2.5.2 Ecological features

Although *Thyasira* cf. *gouldi* and *T. plana* often co-occur, their relative abundance appears site-specific, and may be related to reduced sulfur availability, temperature, organic matter content and/or sediment grain size (Batstone & Dufour 2016). The presence of bacterial symbionts in *T.* cf. *gouldi* (confirmed in both Bonne Bay and the Arctic sites) could suggest that this species preferentially colonizes sediments with greater organic matter content and reduced sulfur content (i.e., abundant energy source for the symbionts), whereas lower tolerance to reduced sulfur might characterize asymbiotic *T. plana*. For instance, the predominance of *T.* cf. *gouldi* at Saglek Bank, a purported hydrocarbon seep (Jauer & Budkewitsch 2010; notably, we observed an oily sheen at the ocean surface during sampling), could be explained by high concentrations of reduced sulfur at this location. Thyasirids often colonize methane seeps (Dando *et al.* 1991; Dando *et al.* 1994; Oliver & Sellanes 2005; Kharlamenko *et al*. 2016; Åström *et al.* 2017) as well as hydrocarbon-enriched sediments near oil fields (Oliver & Killeen 2002), and several studies confirm the presence of bacterial symbionts in those thyasirids (Oliver & Sellanes 2005; Kharlamenko *et al*. 2016). However, *T.* cf. *gouldi* does not appear restricted to

sulfur-rich sediments, but instead appears tolerant of broad range of conditions, including the more restricted ecospace inhabited by *T. plana*. At Bonne Bay, *T.* cf. *gouldi* colonizes three sampling sites, of which the shallowest site, Neddy's Harbour, appears inhospitable to the asymbiotic *T. plana* (Batstone *et al.* 2014, Batstone & Dufour 2016). Intriguingly, sediments at Neddy's Harbour are coarser and less organic-rich than sediments from the two other Bonne Bay sites inhabited by thyasirids (Zanzerl *et al.* 2019). While *T. plana* is much more abundant than *T.* cf. *gouldi* in some locations such as Frobisher Bay, there are currently no known sites where *T. plana* occurs but not *T.* cf. *gouldi* does not.

The different feeding strategies of *T.* cf. *gouldi* and *T. plana* could partly explain the habitat specificity of each species. As mixotrophs, *T.* cf. *gouldi* obtain nutrients both through the digestion of endocytosed symbionts and through particulate feeding, mainly on sedimentary organic matter (Zanzerl *et al*. 2019). *T. plana* appears to feed mainly on suspended particulate organic matter (Zanzerl *et al.* 2019). The more flexible feeding mode of *T.* cf. *gouldi* may allow them to inhabit a broader range of sediment types, including sediments with less reduced sulfur (although their pedal mining and irrigation behaviours could facilitate access to nutrients they or their symbionts require). Furthermore, the symbionts of *T.* cf. *gouldi* show a remarkable degree of metabolic flexibility (McCuaig *et al*. 2020), which could allow them to function under a wide range of environmental conditions. Previous work interprets the relatively broad range in tissue carbon isotope ratios among specimens of *T.* cf. *gouldi* from the Bonne Bay sites as evidence of trophic flexibility, whereas the isotopically lighter nitrogen isotope ratios reflect a greater importance of chemosynthetic bacteria to their diet than in *T. plana* (Zanzerl *et al*. 2019). The results obtained here support these findings, despite notably heavier tissue nitrogen isotope ratios

in Saglek Bank than in Bonne Bay, possibly due to differences in the signature of environmental nitrogen sources rather than dietary differences. Importantly, isotopically heavier nitrogen in the tissues of *T. plana* from Saglek Bank than in *T.* cf. *gouldi* suggests that the interspecific trophic differences previously observed in Bonne Bay also applied to Saglek Bank. Across sampling sites, *T. plana* appears to have a more restricted diet than *T.* cf. *gouldi*, and may require more specific environmental conditions, such as a more constant supply of suspended organic carbon. In Bonne Bay, *T. plana* occurs at sites with considerable terrestrial matter inputs; the predominance of *T. plana* in Frobisher Bay could reflect similar terrestrial influence.

The examination of shell carbonate isotope ratios, which reflect growth rates (carbonate ^{13}C is depleted more rapidly in faster growing bivalves, Dando & Spiro 1993), suggests differences in growth rates between the two species and between sampling sites. At both sampling sites, greater intraspecific variation in shell carbon isotopic ratios for *Thyasira plana* than for *T.* cf. *gouldi* suggests greater variability in growth rates in *T. plana*. The presence of symbionts in *T.* cf. *gouldi* may buffer this species against changes in external food variability (Mariño *et al.* 2019) and support a more constant growth rate than in asymbiotic species. Growth rates in *T. plana* might be especially variable in Saglek Bank, the higher latitude location that presumably experiences greater seasonal variability as well as greater small-scale habitat heterogeneity in this presumed hydrocarbon seep. For *T.* cf. *gouldi*, we found some evidence for slightly faster growth (lighter shell carbonates) at the Bonne Bay site, possibly due to the higher temperatures at the lower latitude site. However, *T.* cf. *gouldi* (and *T. plana*) appear to reach a larger size at higher latitudes, perhaps suggesting a longer lifespan in colder waters.

2.5.3 Geographic range and a possible "*Thyasira gouldi*" species complex

Thyasira cf. *gouldi* and *T. plana* are currently known to occur at three locations along the coast of Eastern Canada. The Labrador Current provides a plausible means of southward dispersal from Arctic locations to Bonne Bay, NL, possibly enabling connectivity among the known populations. Although described from several localities in European waters and the Russian Arctic, it is notable that the sequence available from a specimen identified as *T. gouldi* from Firth of Forth, UK (accession number AJ581871.1) is genetically distinct from *T.* cf. *gouldi* and *T. plana*. Notably, Gould (1841) had considered that specimens of *T. gouldi* (at that time designated as *Lucina flexuosa*) from British locations were identical to the American specimens from the type location, but Philippi (1845) reported considerable differences in shell outline and size between European and American specimens. Philippi's observations subsequently led Gould & Binney (1870) to recognize the European and American specimens as separate species. *T. gouldi* likely includes a cryptic species complex (including the two taxa described herein) rather than a single species with a circumboreal, subarctic distribution. The proper delineation of species within this complex requires careful examination of multiple morphological and genetic characters across a range of geographical locations.

2.6 Conclusions

Based on genetic analyses and the examination of morphological characters, we "resurrected" a species, *Thyasira plana,* which is asymbiotic and distinct from *T. gouldi* s.s. We designated other symbiotic specimens from Bonne Bay and Arctic locations as *T.* cf. *gouldi*, and

further studies may indicate that they form a separate species from *T. gouldi* s.s. Both *T. plana* and *T*. cf. *gouldi* are known from fjords and a possible seep, sites with organic-rich sediments. *T. plana* and *T*. cf. *gouldi* differ from each other slightly in shell outline and in the location of a ferruginous patch on the dorsal end of the shell, and more markedly in the degree of development of gill filaments (observable in thin section) and in the presence or absence of symbionts. We collected specimens of both taxa from three sites, and demonstrate, for the first time, the presence of bacterial symbionts in thyasirids (*T.* cf. *gouldi*) from the Canadian Arctic. *T*. cf. *gouldi* and *T. plana* have a smaller prodissoconch than *T. gouldi* s.s., and their prodissoconch increases in size with latitude. This work suggests that the species *T. gouldi*, as previously defined, encompasses a complex of cryptic species (some with and some without symbionts) that requires further attention. We also highlight the usefulness of larval shell size for thyasirid identification, but caution that proper diagnosis must take latitude into account.

2.7 References

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Chapter 3: Observations of the identity and distribution of thyasirid bivalves in the Canadian Arctic and Eastern Canada

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3.1 Abstract

The Thyasiridae family consists of small cold-water marine bivalves found in sediments globally, often abundant in organically enriched substrates (e.g. fjords, methane seeps). The family is particularly interesting in that it includes both species that form nutritional chemosymbioses with sulphide-oxidizing bacteria and species that lack such bacteria. The burrowing behaviour of symbiotic thyasirids re-oxygenates sediments after organic enrichment events (e.g. oil spills, whale falls, aquaculture sites) which may serve as important ecological functions in the recovery of these unique environments. Therefore, the Thyasiridae may serve as a valuable indicator in sediment remediation but only after proper identification of species. Ambiguous shell features and the lack of taxonomic attention across large geographic areas add to the challenge of identification of thyasirids. Many thyasirids from Canada, most notably in northern regions, have been assigned the names *Thyasira gouldi* and *Thyasira flexuosa*. Both species have created frequent confusion and considerable comment on their similarity. Such taxonomic issues impact museum or other reference collections, causing confusion for benthic

researchers attempting to identify thyasirid species. In addition, poor descriptions of the soft anatomy of many thyasirid species (e.g. *T. gouldi*, *T. flexuosa*, *Thyasira equalis*, *Thyasira dunbari*), particularly for specimens from type locations, has resulted in descriptions of many thyasirid species based solely on shell characters. Genetic data has improved thyasirid species identification, but noting difficulty in locating properly persevered material for molecular analysis.

Here, we provide new information for thyasirid taxa (*Thyasira* cf. *gouldi*, *Thyasira plana*, *T.* cf. *dunbari*, *T.* cf. *equalis* and *Axinopsida orbiculata*) commonly found in the Canadian Arctic and Eastern Canada. This information includes shell measurements, internal and external morphological characters, new gene sequences for *T.* cf. *equalis* and *T.* cf. *dunbari,* distribution records for examined specimens, and descriptions outlining key identifying characters. Our findings aim to improve the classification of the thyasirids from these regions, thereby assisting biodiversity and ecological studies, habitat assessment, and environmental monitoring.

3.2 Introduction

The Thyasiridae is a family of small (generally less than 10 mm in length), cold water-adapted bivalves that occur in sediments along the Canadian coastline. The globally distributed family lives infaunally within various types of substrates (e.g. mud, sand) from coastal to hadal depths (Payne & Allen 1991). Thyasirids are often particularly abundant in organically enriched environments (e.g. whale falls, fjords, hydrothermal vents, oil spills) (Oliver & Killeen 2002; Oliver & Holmes 2006). The family has received particular interest because it includes species

that establish nutritional chemosymbioses with sulphide-oxidizing bacteria maintained at the surface of their gills, as well as species that lack such symbionts (Dando & Southward 1986; Southward 1986; Dufour 2005). Symbiotic thyasirids may provide particular ecological functions, such as re-oxygenating sediments after natural or anthropogenic organic enrichment events (e.g. methane seeps, aquaculture sites) (Oliver & Killeen 2002; Dando *et al.* 2004, Taylor & Glover 2010); as such, they could serve as valuable indicators of organic enrichment or recovery. However, use of thyasirids as effective ecological indicators requires proper identification of species. More thorough descriptions of the characteristics of co-occurring species can facilitate thyasirid species identification, alongside more accurate distribution maps.

Species descriptions mainly based on relatively ambiguous shell features as well as a lack of taxonomic attention across large geographic areas adds challenge to the identification of thyasirids (Oliver & Killeen 2002). Some regionally-focused taxonomic guides have carefully described and delineated thyasirid species (e.g. Payne & Allen 1991; Oliver & Killeen 2002, Coan *et al*. 2000). Researchers have assigned many thyasirids from Canada, particularly in northern regions, to two "catch-all" species with reportedly broad distributions in the Arctic, North Atlantic and North Pacific: *Thyasira gouldi* (Philippi, 1845) and *Thyasira flexuosa* (Montagu, 1803) (species-specific records accessed from obis.org in July 2022). Both *T. gouldi* and *T. flexuosa* were described as symbiotic with shells of variable shape (Oliver & Killeen 2002; Duperron *et al.* 2013). Authors have commented on the similarity and frequent confusion of *T. gouldi* with *T. flexuosa* (Oliver & Killeen 2002), and some consider the two species as synonyms (e.g. Coan *et al.* 2000). Notably, Ockelmann (1958) recognized that *T. flexuosa* was absent from Greenland waters, which suggests that species are distinct and may not overlap

entirely in their distribution. Such taxonomic ambiguities may impact museum or other reference collections, causing confusion for benthic researchers attempting to identify thyasirid species.

Poorly described soft anatomy of many thyasirid species, e.g. *T. gouldi*, *T. flexuosa*, *T. equalis* (Verrill & Bush, 1898), particularly for specimens from type locations, has contributed to many descriptions of thyasirid species being solely based on shell characteristics. Genetic data have helped in establishing phylogenetic relationships among thyasirid species (Taylor *et al*. 2007) and in bringing attention to cryptic diversity in the family (Batstone *et al*. 2014). Nonetheless, the low number of available gene sequences for thyasirids has limited the use of gene sequences to improve species identifications (22 species in GenBank as of January 2023, with nuclear genes available for 15 species, and mitochondrial genes available for 15 species). The difficulty in locating properly preserved thyasirid material for molecular analysis adds further challenge, most notably in specimens preserved in formaldehyde.

Thyasira dunbari Lubinsky, 1976 also occurs in the Canadian Arctic, originally described from latitudes > 75˚ in 10-70 m depth in fjords and bays of the central Canadian Arctic Archipelago (Lubinsky 1976). *T. dunbari* has been reported as a high Arctic species (Aitken & Gilbert 1996; Węsławski *et al.* 2003; Włodarska-Kowalczuk & Pearson 2004; Åström *et al.* 2018), endemic to the Canadian-Greenlandic region (Lubinsky 1976; Oliver & Killeen 2002). The lack of soft tissue and genetic data from the type location has led to the identification of this species based solely on shell characters, including an asymmetrical shell higher than long (H:L = 1.16–1.29), with a massive callus associated with the hinge (Lubinsky 1976). Notably, the description is based upon relatively large specimens (holotype and paratype shell lengths range from 6.1–6.5 mm and shell heights range from 7.8–8.6 mm), with two smaller specimens (5 mm

height) described as having rounded and more symmetrical shells (H:L ratios = 1.02 and 0.96; Lubinsky 1976). Importantly, the two smaller specimens of *T. dunbari* imaged and described in Lubinsky (1976) resemble *T. equalis*, which leads to confusion when attempting to distinguish them (Oliver & Killeen 2002). *T. equalis* reportedly has a wide distribution, from the east coast of the US (Verrill & Bush 1898; WoRMS Editorial Board 2022) and Canada (see Appendix A) to Scandinavian European waters (Oliver & Killeen 2002; Taylor *et al.* 2007), but is not considered to be an Arctic species (Oliver & Killeen 2002). Some authors have reassigned both *T. dunbari* and *T. equalis* to the genus *Parathyasira* (Payne & Allen 1991; Oliver & Killeen 2002; WoRMS Editorial Board 2022) because both taxa share a characteristic shell feature, the sharp submarginal sulcus, and lack an auricle, which is considered to be diagnostic of this genus (Oliver & Killeen 2002). Noting some doubt regarding the validity of the genus *Parathyasira* (Huber 2015; Oliver 2015; Oliver & Rodrigues 2017; Kamenev 2020), we use the genus *Thyasira* in reference to these species, unless referring to published gene sequences.

For this study, we chose a conservative approach in assigning specimens to existing taxa. We identified many specimens as *T.* cf. *dunbari* because we could not find sufficient arguments to show convincingly that they belonged to a new, undescribed species. We provide new genetic and soft anatomy data for *T.* cf. *dunbari* based on specimens obtained close to the type location of *T. dunbari*. For *T. equalis*, many of our specimens varied slightly in shell characters when compared to shells of type specimens, therefore we suspect cryptic diversity within this group. Hereafter, we use *T.* cf. *equalis* for specimens examined in this study, in the absence of sufficient data (genetics, soft tissue) from the type location.

Here, we provide new information for thyasirid taxa (*Thyasira* cf. *gouldi, Thyasira plana, T.* cf. *dunbari, T.* cf. *equalis* and *Axinopsida orbiculata* (Sars, 1878)) commonly found in the Canadian Arctic and Eastern Canada, which include: measurements, internal and external morphological characters, new gene sequences for *T.* cf. *equalis* and *T.* cf. *dunbari*, geographic distribution, and descriptions outlining key identifying characters. Our analysis is based on: 1) newly sampled thyasirids from the CCGS *Amundsen* cruises in 2016 and 2017 to Baffin Bay; and 2) the CMN collections, limited to the Canadian Arctic and Eastern Canada. Where possible, we include information from type specimens or locations. We aim to improve the classification of thyasirids from these regions, thereby assisting biodiversity and ecological studies.

3.3 Materials and Methods

3.3.1 Specimens Examined

During the CCGS *Amundsen* leg 2 cruise in July 2016, *Thyasira cf. gouldi* and *Thyasira plana* were collected from regions within northernmost Baffin Bay: inner and outer Frobisher Bay (63°38’N, 68°37’W and 62°55’N, 67°05’W, respectively; depth range: 62–610 m) and Saglek Bank (60°18’N, 62°11′W; depth 275 m). During the CCGS *Amundsen* leg 2b cruise in July 2017, *Thyasira* cf. *dunbari* and *Thyasira* cf. *equalis* were collected higher north of Baffin Bay (74°09’N, 80°28’W; 76°19’N, 71°15’W; 76°18’N, 73°12’W; depth range: 593–787 m). Specimens were collected on the CCGS *Amundsen* using a 0.25 m^2 box corer and sieved (1 mm mesh) to retrieve thyasirids: N=12 from 2017 and N=62 from 2016 were preserved in 2.5% glutaraldehyde in filtered seawater (as in Montanaro *et al*. 2016); N=20 from 2017 and N=55

from 2016 were preserved in 95% ethanol; N=6 from 2017 and N=17 were frozen at -20 °C for morphological and genetic analyses.

In 2016 and 2019, we examined wet and dry thyasirid faunal collections (N=30 in 2016 and N=32 in 2019) from the Canadian Museum of Nature in morphological detail. We include measurements and identifications from these collections in this study for the species *T.* cf. *gouldi* (N=18)*, T. plana* (N=38), *T.* cf. *dunbari* (N=19), *T. equalis* sensu stricto (N=2), *T.* cf. *equalis* (N=8) and *Axinopsida orbiculata* (N=10) (see Appendix A).

We also included morphological analysis on species *T.* cf. *gouldi* (N=43) and *T. plana* (N=17), collected in 2011 using a Peterson grab (12 cm diameter/sampling depth) from Bonne Bay, Newfoundland (49°30′N, 57°55′W).

Additional specimens of *Axinopsida orbiculata* (N=7) from Frobisher Bay (63°43′N, 68°31′W) were collected by Erin Herder using a Van Veen grab (surface area 0.111 m^2) on the M.V. *Nulialjuk* in October 2016. Specimens were fixed in 10% buffered formalin for 24 h prior to transfer to 70% ethanol for preservation (Herder, 2020).

3.3.2 Morphological Observations and Measurements

For specimens collected during the CCGS *Amundsen* leg 2 cruise in 2016 and Bonne Bay in 2011, we imaged the right and left external valve, internal and external anatomy (where possible), prodissoconch (the umbonal region) and sub-marginal sulcus (SMS) on a Leica MZ95 and an Olympus SZ 61 dissecting microscope. We determined prodissoconch length (to the nearest 1 μm) and shell dimensions (to the nearest 0.1 mm) using the measuring tool in ImageJ (Abramoff *et al*. 2004). For specimens examined at the Canadian Museum of Nature, we imaged

of the same anatomical characters listed above on an Olympus SZX12 dissecting microscope, and obtained measurements using Pixelink uScope Professional.

3.3.3 18S and 28S rRNA DNA extraction

We obtained tentatively identified *T.* cf. *dunbari* specimens from the Canadian Museum of Nature for DNA sequencing attempts. Specimens collected by Dr. Virginie Roy in 2010 from Lancaster Sound (74°11'19" N, 83°58'25" W) and 2011 from Dease Strait (68°59'58" N, 106°33' 38" W) were fixed in 10% formalin and then preserved 70% ethanol. In addition, we sequenced specimens collected in 2017 from higher north of Baffin Bay (76°19'N 71°15'W, 76°18'N 73°12'W). DNA was isolated from thyasirid gills (N=5 Dease Strait, N=2 Baffin Bay) using the QIAgen DNeasy Blood & Tissue Kit, following the spin-column protocol for animal tissues. We performed polymerase chain reaction (PCR) amplifications using gene-specific sets of primers as in Batstone *et al*. (2014): 1) 18S-5' (Winnepenninckx *et al.* 1998) and 18S1100R (Williams *et al.* 2003) for a ~1000 base pair (bp) fragment of the 18S rRNA gene; 2) LSU-59 (Littlewood *et al.* 2000) and LSU1600R (Williams *et al.* 2003) for a ~1500 bp fragment of the 28S rRNA gene. The PCR mixture and thermocycler conditions followed McCuaig *et al.* (2017). Our PCR attempts yielded no product for Dr. Roy's samples, so we subjected the isolated DNA to a repair step using the NEBNext FFPE DNA Repair Mix (New England Biolabs). Our reattempted PCR was unsuccessful.

3.3.4 Light and Transmission Electron Microscopy

Selected thyasirid gills (N=6) dissected out of specimens obtained from the Canadian Museum of Nature were fixed in 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer for 24 h, post-fixed in 1% osmium tetroxide in the same buffer for 1 h, dehydrated in an ascending

ethanol series, and embedded in EPON resin (Batstone *et al.* 2014). Semi-thin (1 μm) sections made using a LKG Bromma 8800 ultramicrotome were stained for approximately 1 minute on a heating plate with 1% toluidine blue in 1% sodium borate for examination through light microscopy. We then took digital micrographs of each gill filament at 200X magnification on a Zeiss Axioscope A1.

Two specimens of *T.* cf. *gouldi* and one of *T. plana* from Saglek Bank, collected during the CCGS *Amundsen* leg 2 cruise (July 2016) and three CMN specimens, *T. cf. equalis* and *T. cf. dunbari*, both from Baffin Bay and *A. orbiculata*, from the Labrador Sea, were immediately fixed in 2.5% glutaraldehyde in filtered seawater (Montanaro *et al*. 2016) and prepared for transmission electron microscopy (TEM). After post-fixation in 1% osmium tetroxide, dehydration in an ascending ethanol series and embedding in EPON resin, we mounted ultra-thin (70 nm) sections on copper grids and stained them with uranyl acetate and lead citrate. Imaging was performed on a FEI Tecnai Spirit TEM operating at 80kV.

3.4 Results

3.4.1 Species Observations

From the specimens examined, we identified 5 thyasirid taxa: *T.* cf. *gouldi, T. plana*, *T.* cf. *dunbari*, *T.* cf. *equalis* and *Axinopsida orbiculata* (Fig. 3.1). Taxa were differentiated based on defining characters, described below, and we mapped the geographic distribution of each taxon, based on the specimens examined. For *T.* cf. *equalis* and *T.* cf. *dunbari,* we present new gene sequence data (see Table 2.3 for GenBank accession numbers). Hereafter we provide brief descriptions of the taxa, focusing on characters we find most useful for discrimination.

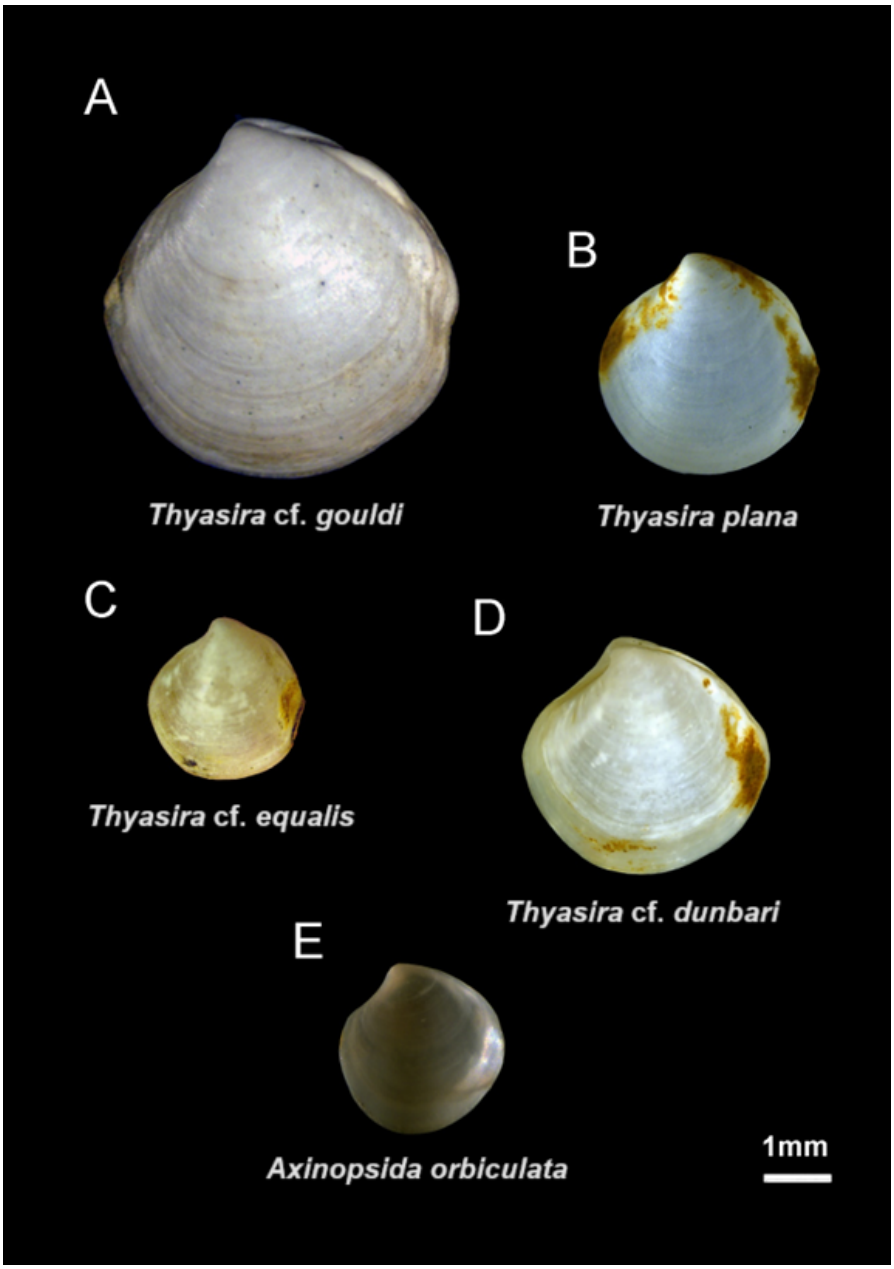

Figure 3.1. Five thyasirid taxa in the study region. All shells are from the Canadian Museum of Nature, showing the left valve in external view. **A,** *Thyasira* cf. *gouldi*, from Hudson Bay, catalog number CMNML 36479. **B,** *Thyasira plana*, from Richmond Gulf, catalog number CMNML 41258. **C,** *Thyasira cf. equalis*, from Cape Walsingham-Holsteinborg, catalog number CMNML 66591. **D,** *Thyasira* cf. *dunbari*, from St. Lawrence River, catalog number CMNML 73960. **E,** *Axinospida orbiculata*, from Foxe Basin, catalog number CMNML 45579.

3.4.2 *Thyasira* cf. *gouldi*

Description: Maximum size, 5 mm (Chapter 2, Table 2.4); fragile; equivalve. Outline subequilateral to roundly-subovate; height slightly greater than or equal to length; umbos projecting, beaks slightly to the anterior; anterior expanded laterally. Lunule margin long and a little sunken; anterior-ventral margin narrowly rounded; posterior margin bisinuate. Posterior area has two distinctly rounded folds and a sharp posterior sulcus (Chapter 2, Fig. 2.7A). Hinge has a weakly defined small tooth in right valve; left valve has a corresponding small depression. White shell; small ferruginous patches near the posterior sinus (Chapter 2, Fig. 2.7A), and the anterior lunule area (Chapter 2, Figs. 2.7C–E). Prodissoconch (Chapter 2, Fig. 2.7B) medium, 155–238 µm in length. Larval shell length increases in size as the latitude increases (Chapter 2, Table 2.4). Foot vermiform with bulbous tip (Chapter 2, Fig. 2.8B). Anterior adductor large and long; posterior adductor short and oval (Chapter 2, Fig. 2.8A). Ctenidium with two demibranchs, thick and fleshy lamellae (Chapter 2, Fig. 2.8B). Lateral body pouch lobed and branched, distinct (Chapter 2, Fig. 2.8B).

Growth changes: The anterior expanding laterally often more visible in adults (Chapter 2, Fig. 2.7). Bisinuation more developed and defined in adults than juvenile specimens (Chapter 2, Figs. 2.7A, C–H).

Variations: Some specimens with length slightly greater than height. Tooth sometimes difficult to locate. Ferruginous patches sometimes absent.

Distribution: A widespread thyasirid in the Canadian Arctic (Baffin Bay) and Eastern Canada (Hudson Bay, Labrador Sea) in depths from 15 to 610 m (see Fig. 3.2).

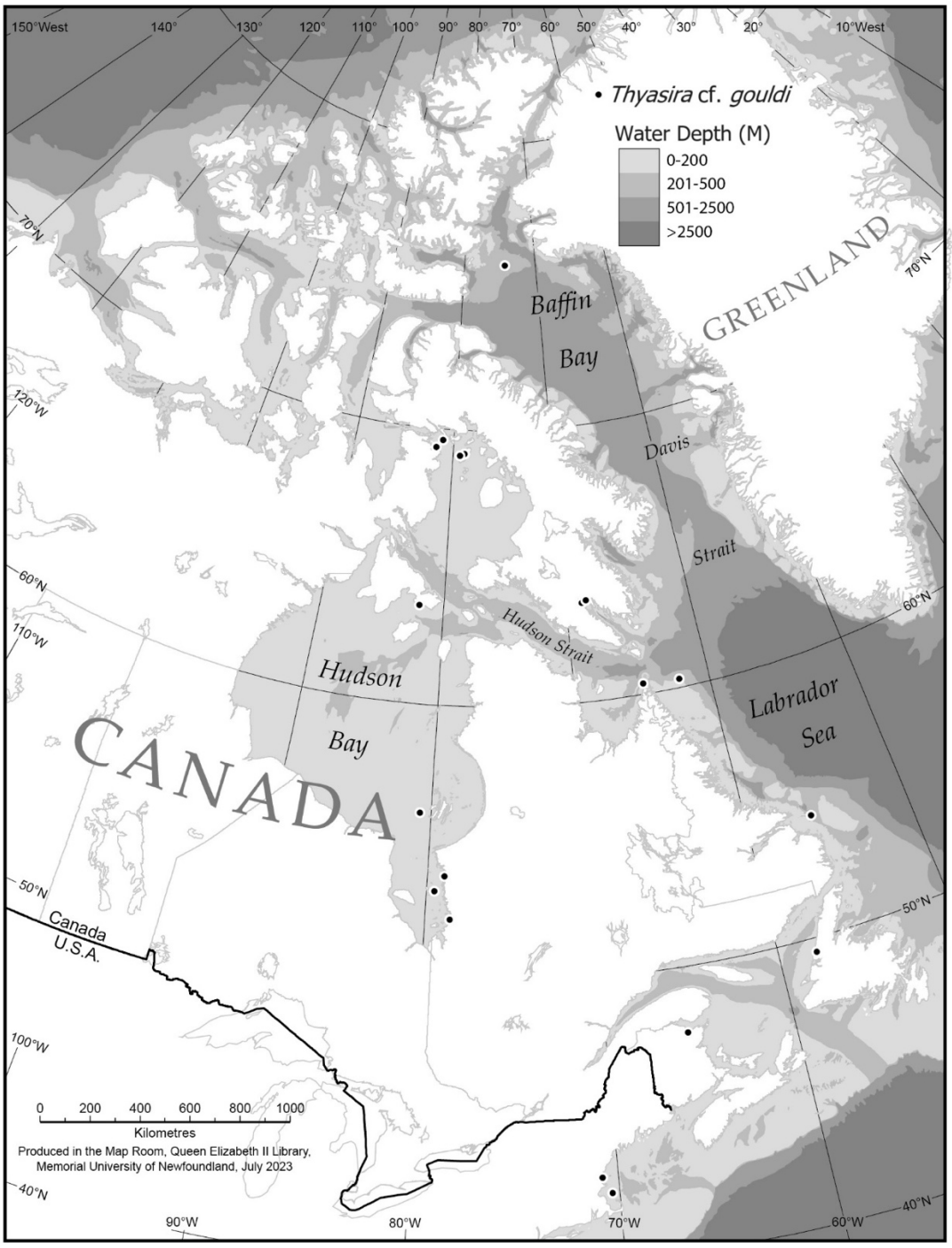

Figure 3.2. Distribution of *Thyasira* cf. *gouldi* in the Canadian Arctic and Eastern Canada. Location near Massachusetts is the type location of *T. gouldi*.

3.4.3 *Thyasira plana*

Description: Maximum size, 3.9 mm (Chapter 2, Table 2.5); thin shelled; equivalve. Outline subequilateral to subovate; beaks towards the anterior; length greater than or equal to height; anterior expanded slightly laterally. Lunule margin moderately long, a little sloping and not sunken; anterior-ventral margin forming a continuous curve; posterior margin weakly bisinuate. Posterior sulcus and submarginal sulcus shallow. Hinge has a weakly defined small tooth in right valve; left valve has a corresponding small depression. White shell; ferruginous patches often visible near posterior sinus (Chapter 2, Figs. 2.9C, G), sometimes expanded laterally along the posterior and anterior area (Chapter 2, Figs. 2.9A, G, I, J). Prodissoconch small-medium, 130–219 μm in length. Larval shell increases in length as latitude increases (Chapter 2, Table 2.5). Foot vermiform with bulbous toe (Chapter 3, Fig. 3.8C). Anterior adductor large and long; posterior adductor short and oval (Chapter 2, Fig. 2.8D). Ctenidium with two demibranchs (Chapter 2, Fig. 2.8C). Lateral body pouch has few lobes and is slightly branched (Chapter 2, Fig. 2.8D). Description summarized from Chapter 2.

Growth changes: Anterior expanded slightly laterally in adults but not seen in juveniles (Chapter 2, Fig. 2.9). Weak bisinuation more defined in adult specimens (Chapter 2, Fig. 2.9).

Variations: Some specimens with height slightly greater than length. Some juvenile shells only show the marginal sinus (Chapter 2, Fig. 2.9I, J). Posterior sulcus absent in some specimens (Figs. 3.9D, I, J). Tooth sometimes difficult to locate. Ferruginous patches sometimes absent, but not often. Lateral body pouch sometimes not showing any lobes (Fig. 3.8D).

Type locality: North of Cape Cod, in the Gulf of Maine, Casco Bay, Bay of Fundy, and Halifax Harbor. Depth: 14 to 183 m

Distribution: A widespread species in the Canadian Arctic (Baffin Bay) and Eastern Canada (Hudson Bay, Labrador Sea) in depths from 30 to 610 m (Fig. 3.2).

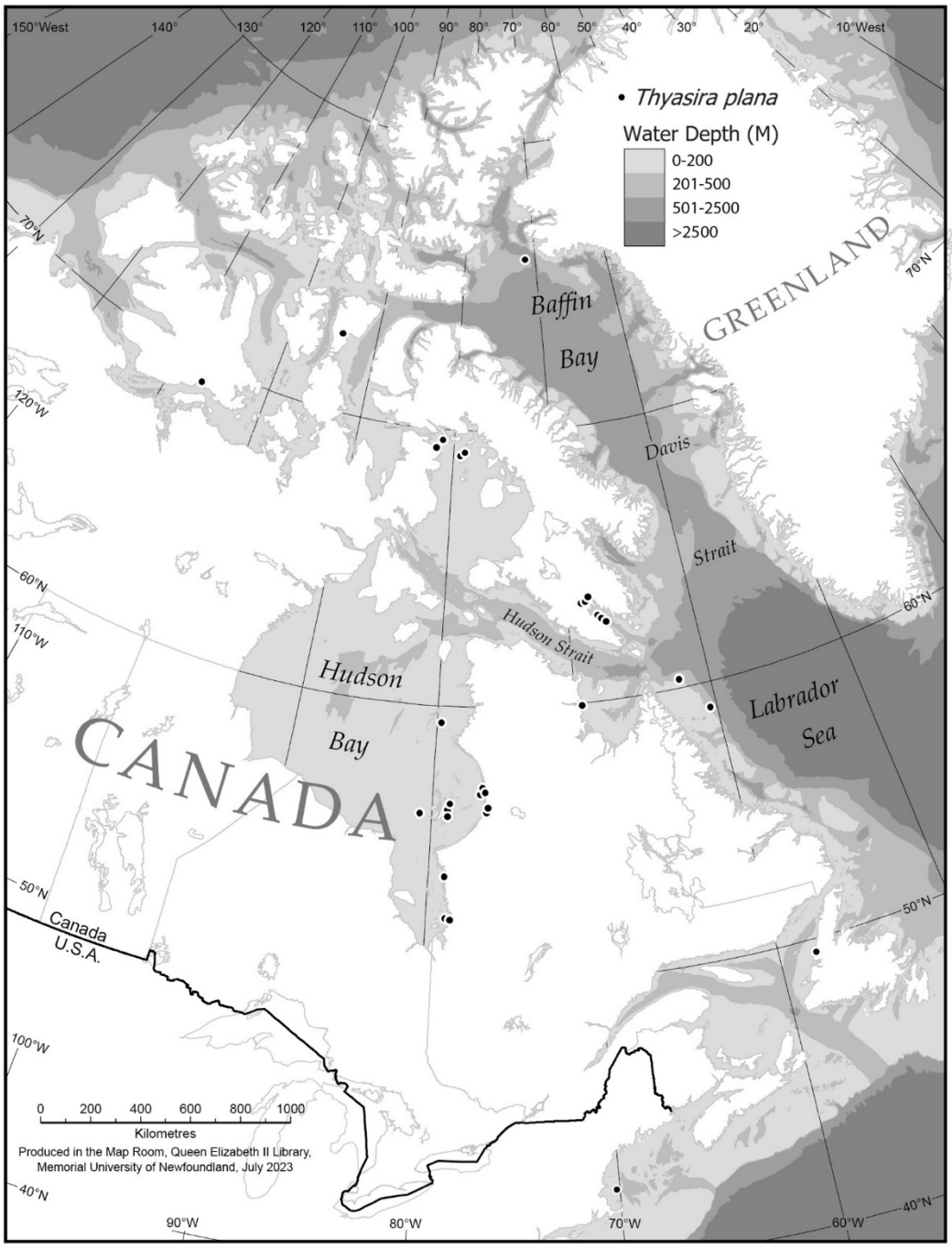

Figure 3.3. Distribution of *Thyasira plana* in the Canadian Arctic and Eastern Canada. Location near Massachusetts corresponds to the syntype location.

3.4.4 *Thyasira* cf. *equalis*

Description: Maximum size, 4.9 mm (Table 3.1); thin shelled; fragile; equivalve. Outline equilateral to ovate-polygonal shaped, higher than long or equal; umbos projecting, beaks slightly prosogyre. Auricle absent. Sub-marginal sulcus long, deep with sharply defined margins (Fig. 3.4C), not forming a marginal sinus. Posterior area has one weakly rounded fold; posterior margin sharply truncated, with varying slight curves (Figs. 3.4A, B, D) or straight (Fig. 3.4G). Ventral margin narrowly rounded. Anterior area round, forming an angulation at junction with lunule. Lunule small; lunule margin short. Hinge weak with a single, very small cardinal tooth in the right valve; left valve has a corresponding small depression below the beak (Fig. 3.5B). White, often transparent shell (Fig. 3.5A); ferruginous patches often visible at posterior area (Figs. 3.4A, B, D, E, 3.5A), sometimes expanding laterally along posterior area (Fig. 3.4F), anterior – lunule margin junction (Figs. 3.4A, B, 3.5A), and sometimes along the ventral margin (Fig. 3.4D). Prodissoconch (Fig. 3.5E) small, 125–144 μm in length (Table 3.1). Ctenidium with two demibranchs, thick and fleshly (Figs. 3.5B, C). Foot vermiform with a bulbous toe (Fig. 3.5C). Lateral body pouch lobed and branched (Figs. 3.5C, D).

Variations: Outline of the posterior margin sometimes shows slight sinuation or is sharply truncated.

Type locality: USFC station 18, Casco Bay, Maine, 172 m.

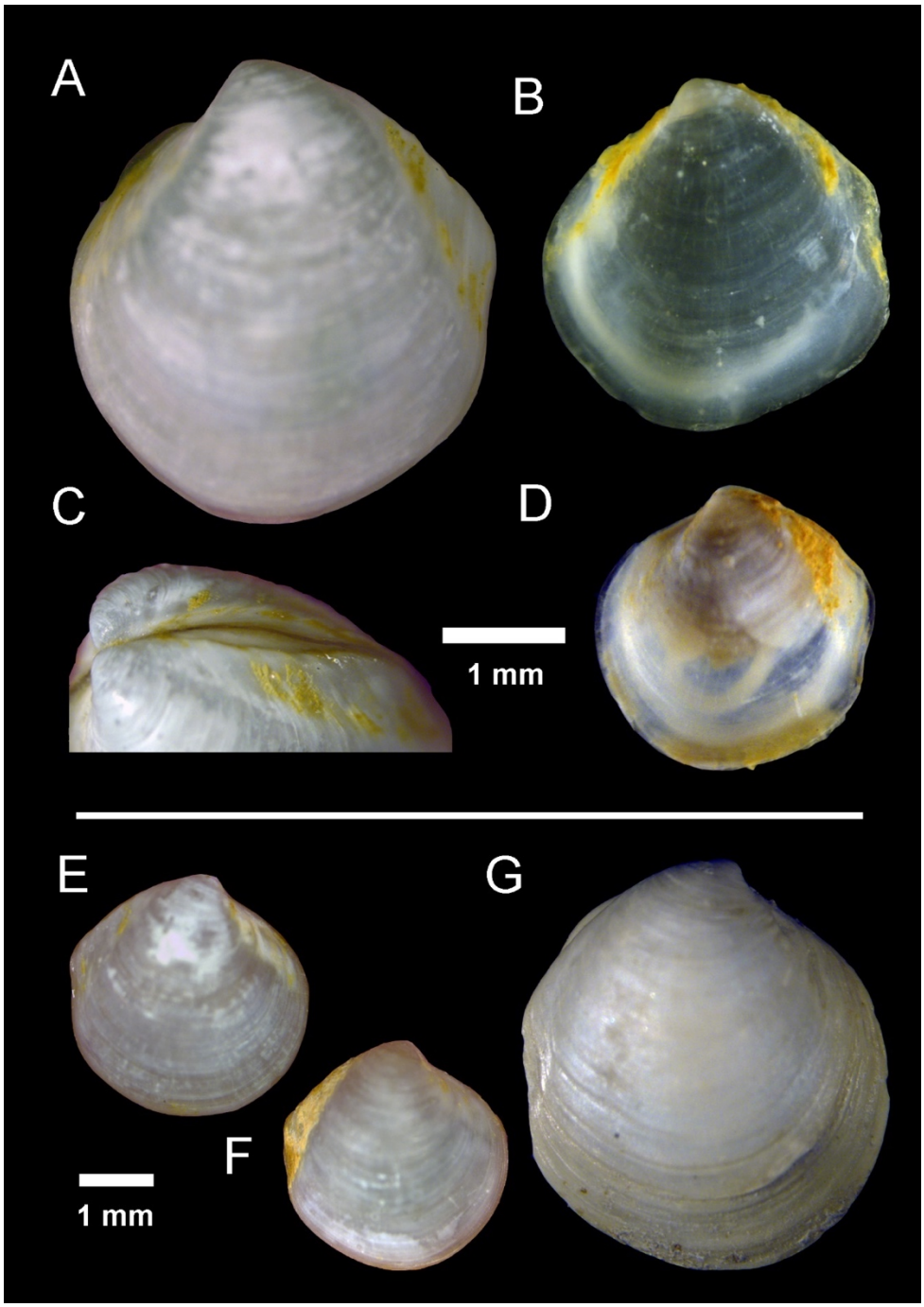

Figure 3.4. ***Thyasira*** **cf.** ***equalis,*** size series of selected specimens from the Canadian Museum of Nature and Baffin Bay. **A, B, D** external left valve. **C,** view of the well-defined sub-marginal sulcus. **E-G,** external right valve. Catalog numbers: **F,** CMNML 66587; **G,** CMNML 45295. **A, C, E:** collected by Dr. Virginie Roy in 2010 from Lancaster Sound. **B, D:** collected by Dr. Bárbara de Moura Neves in 2017 from Baffin Bay.

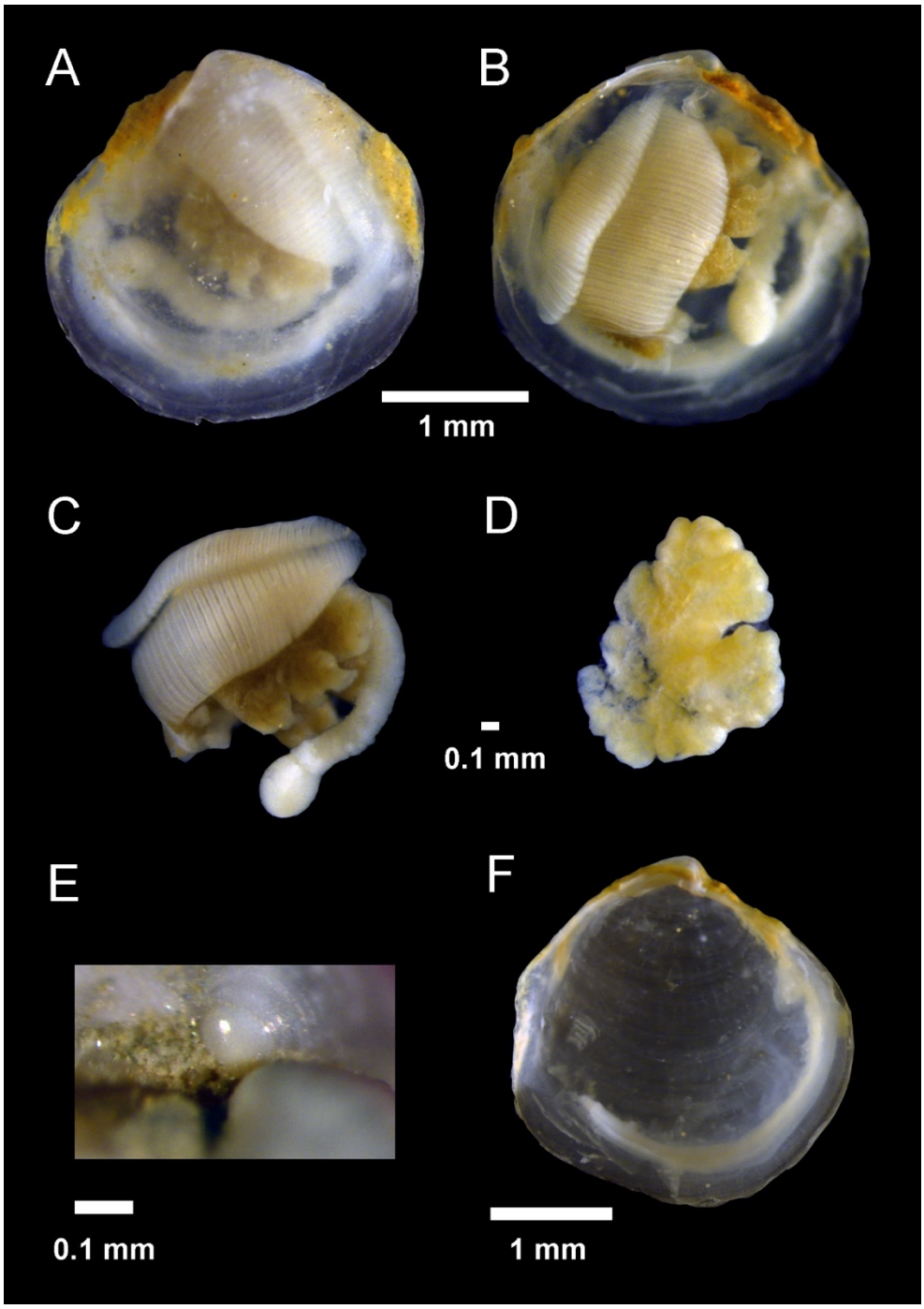

Figure 3.5. ***Thyasira*** **cf.** ***equalis*****,** key anatomy characters. **A,** external left valve. **B,** internal left valve with anatomy. **C,** anatomy. **A-B,** same specimen. **D,** lateral body pouch. **E,** prodissoconch. **F,** internal left valve without anatomy. **D, F,** same specimen. **A-D, F:** collected by Dr. Bárbara de Moura Neves in 2017 from Baffin Bay. **E:** collected by Dr. Virginie Roy in 2010 from Lancaster Sound.

Distribution: Widely found in the Canadian Arctic (Baffin Bay, Davis Strait). Our research shows that it is occurs rarely found in Eastern Canada (Hudson Bay), but further museum examinations are needed. Depths range: 91–931 m (see Table 3.1).

Remarks: Similar to smaller specimens of *T. dunbari* in that the shell does not greatly expand antero-ventrally (Oliver & Killeen 2002).

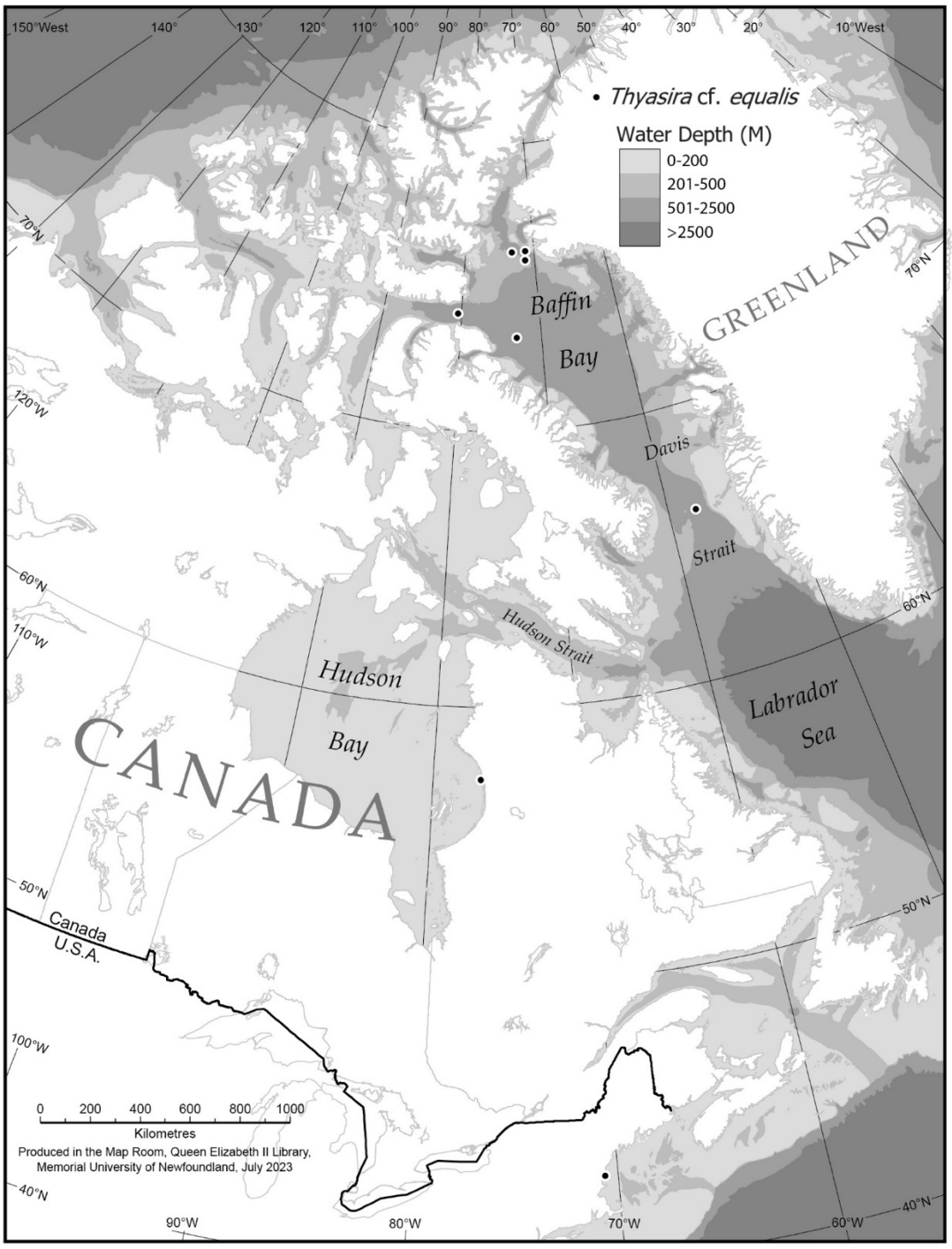

Figure 3.6. Distribution of *Thyasira* cf. *equalis* in the Canadian Arctic and Eastern Canada. Location near Massachusetts is the type location for *T. equalis*.

3.4.5 *Thyasira* cf. *dunbari*

Description: Maximum size, 7–9 mm (Lubinsky 1976); equivalve; thin shelled. Adults outline elongated, expanded antero-ventrally (Figs. 3.7A, B); smaller, more common specimens outline equilateral-subovate (Figs. 3.7E, F); height greater than length; umbos projecting, beaks prosogyre. Lunule margin long and sloping; junction of lunule margin and anterior margin narrowly rounded, sometimes slightly truncated (Fig. 3.7D); anterior-ventral margin rounded and angulated laterally; posterior area poorly defined, long, flattened; posterior margin slightly angular to narrowly rounded. Auricle absent; sharp and well defined sub-marginal sulcus (Figs. 3.7G–I). Hinge with no tooth, similar in both valves (Figs.3. 8A, B). White shell; ferruginous patches sometimes visible along posterior and anterior margins (Figs. 3.7A–C, E) or expand laterally along posterior area (Fig. 3.7F). Prodissoconch (Fig. 3.8B) small-medium, 122–179 μm in length (Table 3.1).

Growth changes: Large specimens distinctly elongate in form, whereas smaller specimens (the species's more typical state, from our observations) equilateral-subovate in outline (Fig. 3.7). Ctenidium with two demibranchs (Figs. 3.8A, C, E), large and fleshly (Fig. 3.8E); foot vermiform with slightly bulbous toe (Figs. 3.8B, C, E). Anterior adductor medium and elongate (Fig. 3.8B); posterior adductor short and oval (Figs. 3.8A–C). Lateral body pouch distinct, extensively lobed and branched (Figs. 3.8C–F); dark brown in color in live-fresh state (Figs. 3.8C, E); eggs sometimes visible (Figs. 3.8A, D).

Variations: Posterior margin can be slightly angular (Figs. 3.7A–C, E) to sharply angular (Figs. 3.7D, F).

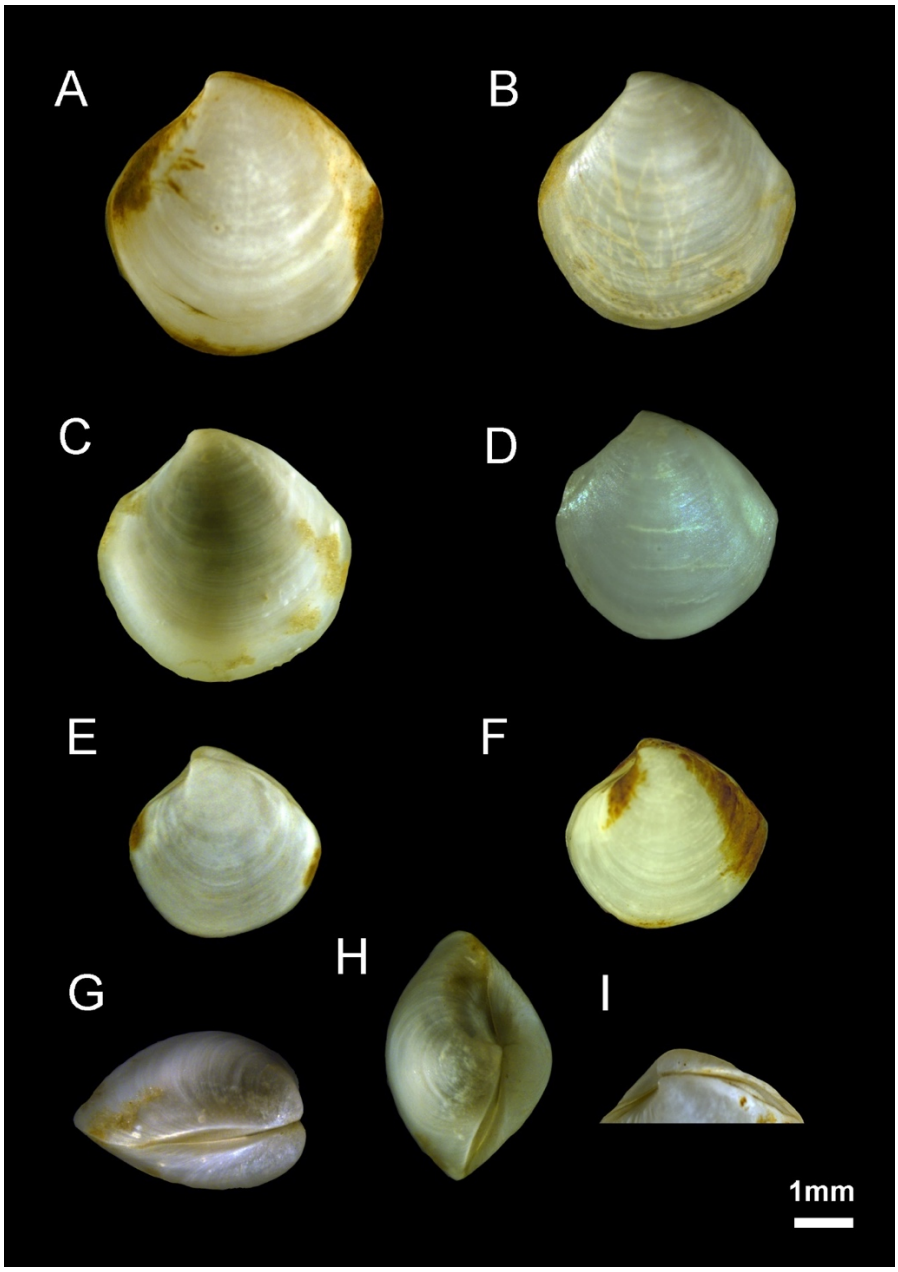

Figure 3.7. ***Thyasira*** **cf.** ***dunbari,*** external shapes, characters, and size series. Specimens from the Canadian Museum of Nature and Baffin Bay. **A,** left valve, from St. Lawrence River, catalog number CMNML 73960. **B,** LV, from Hudson Bay, catalog number CMNML 45343. **C,** LV, from Baffin Bay. **D,** LV, from Bonne Bay, catalog number CMNML 70476. **E,** LV, from Baffin Bay. **F,** LV, from St. Lawrence River, catalog number 73960. **G**–**H,** view of well-defined sub-marginal sulcus, from Baffin Bay. **I,** sub-marginal sulcus. **C, E, G, H:** collected by Dr. Bárbara de Moura Neves.

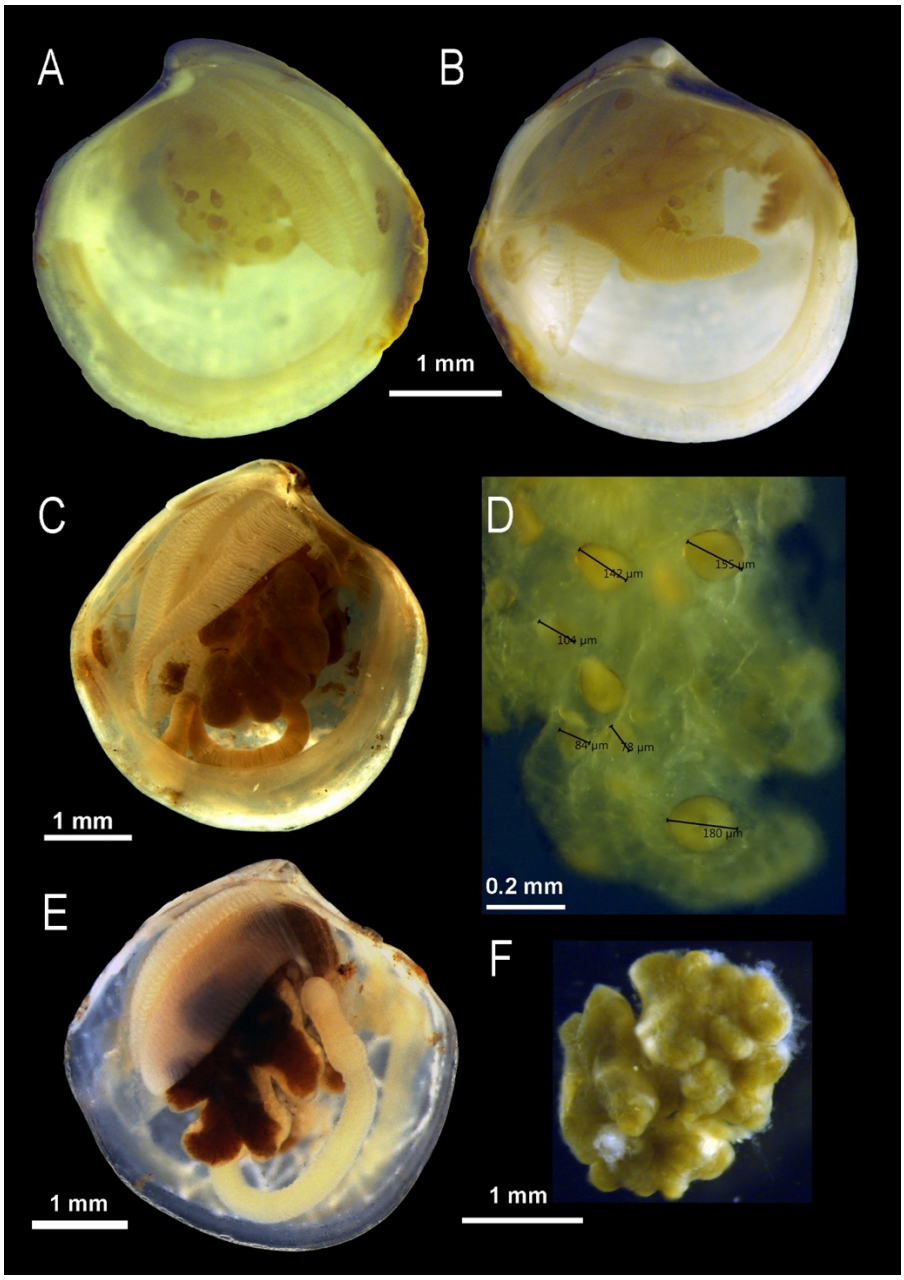

Figure 3.8. ***Thyasira*** **cf.** ***dunbari,*** internal anatomy. **A**–**B: A,** internal right valve with lateral body pouch and gills, **B,** internal left valve with foot, view of larval shell; from Baffin Bay, catalog number CMNML 66593. **C,** freshly sampled internal left valve with anatomy, from Beaufort Sea, collected by Dr. Suzanne C. Dufour. **D,** lateral body pouch with eggs, egg size range: 78–180 µm, from specimen A–B. **E,** freshly sampled internal left valve with anatomy, from Bonne Bay, collected by Dr. Suzanne C. Dufour. **F,** lateral body pouch, from Baffin Bay, specimen collected by Dr. Bárbara de Moura Neves.

Type locality: Hole-in-Fog Bay, Isachsen, Ellef Ringnes Island.

Distribution: The smaller form is not endemic to the high Arctic (Canadian-Greenlandic region) as previously stated (Lubinsky 1976; Oliver & Killeen 2002), but is widespread throughout the Canadian Arctic and Eastern Canada (Fig. 3.9).

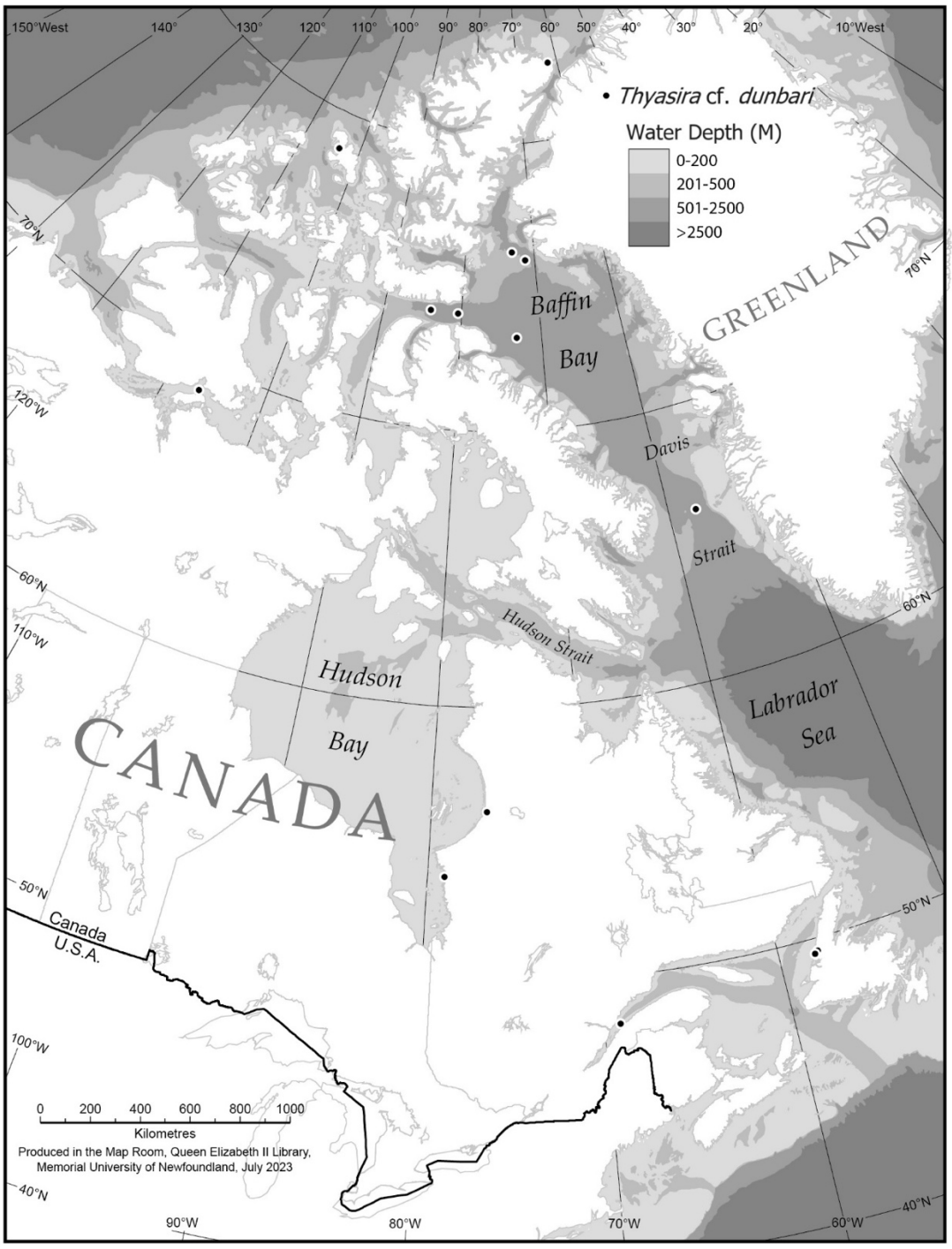

Figure 3.9. Distribution of *Thyasira* cf. *dunbari* in the Canadian Arctic and Eastern Canada.

3.4.6 *Axinopsida orbiculata*

Description: Maximum size, 8 mm in length (Oliver & Killeen 2002); equivalve; equilateral. Outline circular (Figs. 3.10A–D) to subcircular (Figs. 3.10E–G); anterior dorsal margin deeply sunken; posterior dorsal margin gently curved; ventral margins broadly rounded. Distinct lunule (Oliver & Killeen 2002); posterior area slightly flattened, lacking sulci. Right valve hinge has a distinct large cardinal tooth or peg (Figs. 3.11D–E), left valve hinge has a corresponding socket. White shell, ferruginous patches sometimes expand laterally along posterior and anterior areas (Fig. 10E); periostracum thin and yellowish. Prodissoconch (Figs 3.11D–E) large, 260–338 µm in length (Table 3.1). Ctenidium with two demibranchs (Figs. 3.11A–B). Pallial line entire, anterior adductor elongate, posterior adductor short and oval (Figs. 3.11A–C). Lateral body pouch lobed and branched (Figs. 3.11A–C).

Growth changes: Large specimens show a distinct circular form (Figs 3.10A–D), whereas smaller specimens can sometimes be subcircular, slightly extending laterally along the anterior-ventral area (Figs 3.10E–G).

Variations: Outline broadly circular or subcircular (Fig. 3.10).

Type locality: Norway (Vardø – Bodø) (Sars 1878)

Distribution: This species is common throughout the arctic and subarctic (Ockelmann, 1958) (Fig. 3.12).

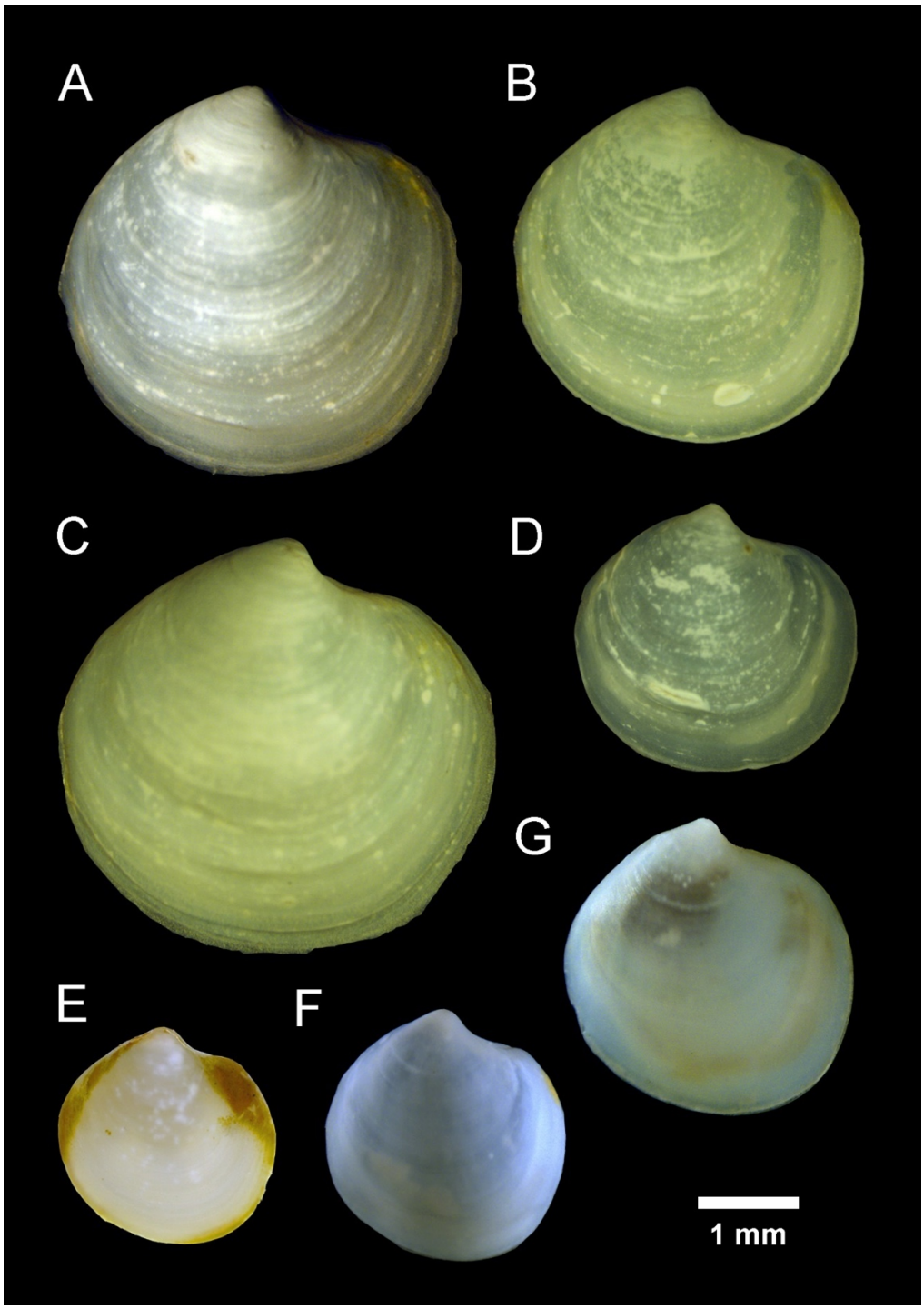

Figure 3.10. ***Axinopsida orbiculata,*** size series of selected specimens from the Canadian Museum of Nature and Frobisher Bay. **A-D,** right valve, from Greenland, catalog number CMNML 43439. **E,** RV, from Frobisher Bay, collected by Erin Herder. **F, G,** RV, from Foxe Basin, catalog number CMNML 45579.

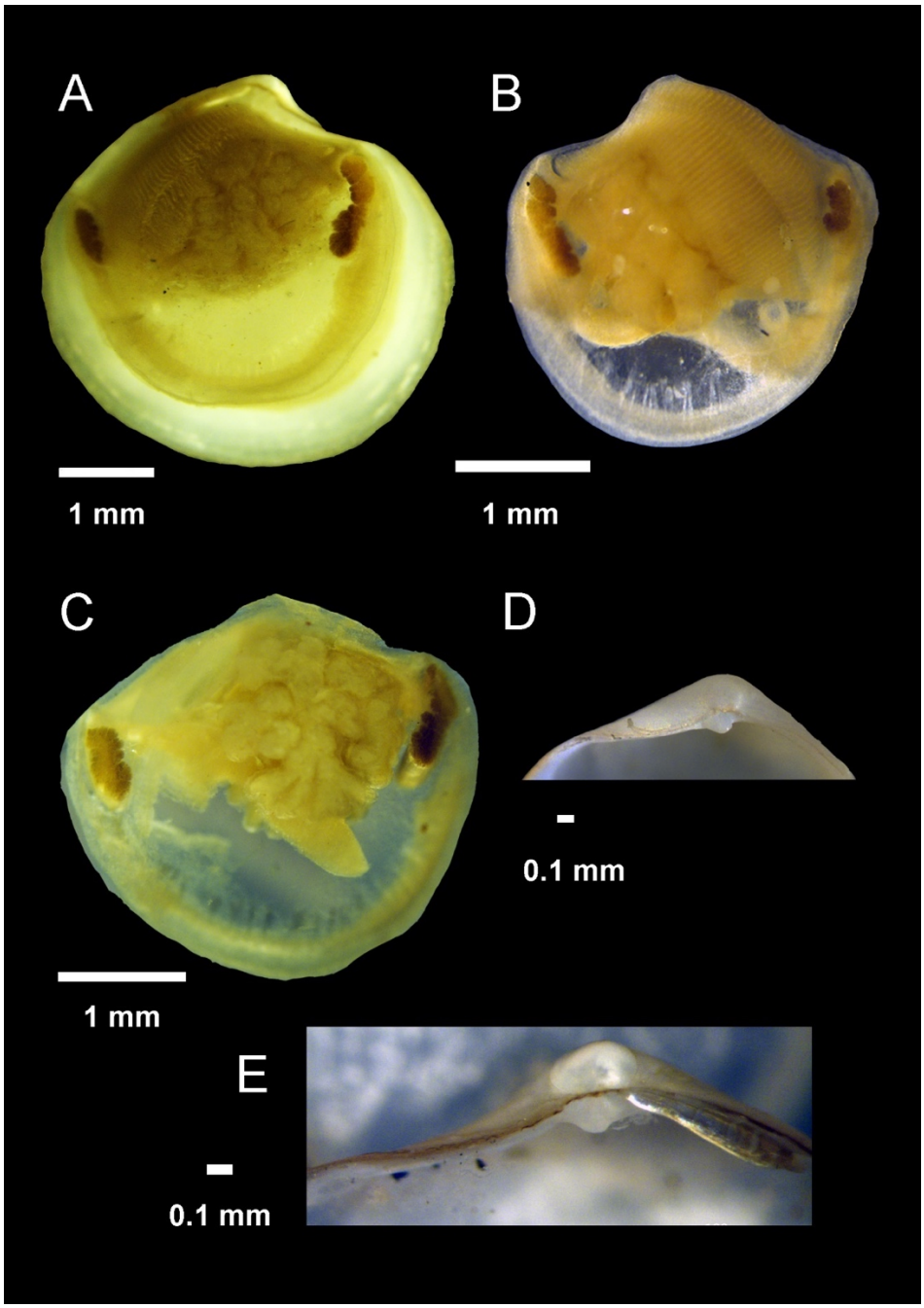

Figure 3.11. ***Axinopsida orbiculata,*** internal anatomy and defining characters. All specimens from the Canadian Museum of Nature. **A,** internal left valve with anatomy, foot absent. **B,** internal anatomy with defined gills. **C,** internal anatomy, branched lateral body pouch, gills absent. **D,** distinct cardinal tooth in right valve, from Foxe Basin, catalog number CMNML 45579. **E,** large prodissoconch: 293 µm, distinct tooth, RV. **A–C, E:** from Greenland, catalog number CMNML 43439.

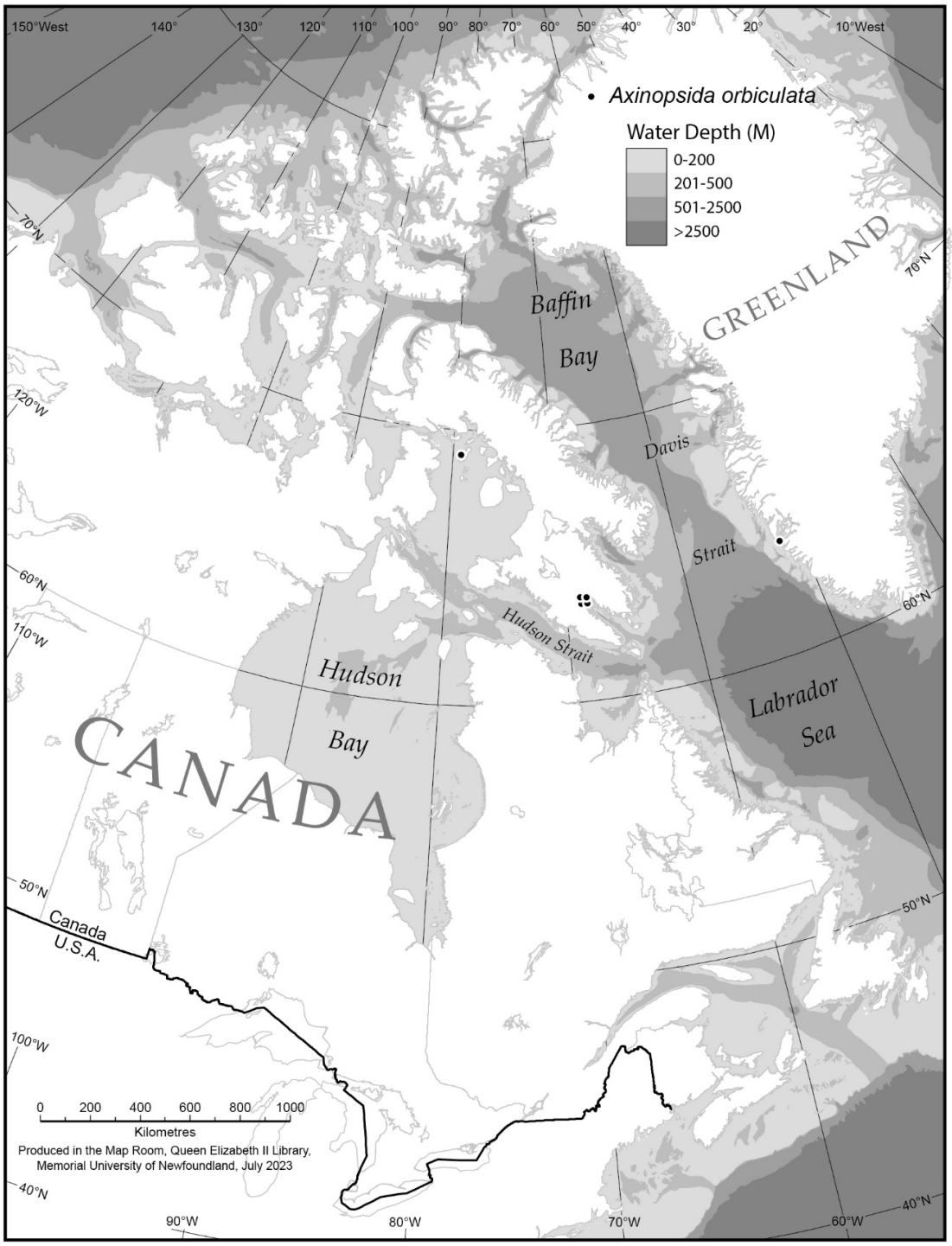

Figure 3.12. Distribution of *Axinopsida orbiculata* in the Canadian Arctic.

Based on specimens examined throughout the Canadian Arctic and Eastern Canada, shell characters and dimensions differ between species; we therefore developed Table 3.1 as a tool for identifying species *T.* cf. *gouldi, T. plana*, *T. dunbari*, *T.* cf. *equalis*, and *A. orbiculata* (Table 3.1). Prodissoconch size was an important character in our identifications, because larval shell sizes are specific to each species, with some overlap (i.e., *T.* cf. *gouldi*: 155–238 μm, *T. plana*: 130–219 μm).

Table 3.1. Shell characters and sampling depths for *T.* cf. *gouldi, T. plana*, *T.* cf. *equalis*, *T.* cf. *dunbari*, and *A. orbiculata* based on our material and publications. Prodissoconch and length measurements are based on specimens from our study; numbers of specimens examined shown in parentheses. See Appendix A for full list of measurements.

Species	Prodissoconch (μm)	Length (mm)	Depth (m)	Submarginal sulcus (SMS)	Tooth (right valve)
Thyasira cf. *gouldi*	155–238 (49)	2.36–5.04 (51)	15–610	Yes, subtle	Small, indistinct
Thyasira plana	130–219 (46)	2.33–3.94 (25)	30–610	Yes, subtle	Small, indistinct
Thyasira cf. *equalis*	125–148 (9)	2.42–4.94 (10)	91–931	Yes, distinct, deep, long[1]	Small, flattened peg[1]
Thyasira cf. *dunbari*	119–179 (19)	2.39–4.72 (15)	7–931	Yes, well defined[1,2]	Toothless[3]
Axinopsida orbiculata	260–338 (12)	2.05–4.67 (13)	0–27	Absent[1]	Large, distinct peg

[1]Oliver & Killeen (2002), [2]pers. comm. Dr. Suzanne Dufour, [3]Lubinsky I. (1976)

3.4.7 Gill Morphology and Symbiont Presence

Symbiont presence can be determined through examination of thin sections of gill filaments (Figure 3.13) using transmission electron microscopy. Of the five taxa observed, only *T.* cf. *gouldi* has symbionts (Figure 3.13), showing an elongated type 3 gill structure (Dufour 2005). Semi-thin sections of gill filament reveal no abfrontal expansion in *T. cf. equalis, T. plana, T.* cf. *dunbari and A. orbiculata* (gill type 2 of Dufour 2005; Figs. 3.13E, F), and no symbionts were observed among abfrontal cell microvilli using transmission electron microscopy (Figs. 3.13D, F, H, J).

3.4.8 Gene sequences

18s rRNA

We obtained 749 bp fragments of 18S rRNA from specimens of *Thyasira* cf. *equalis* and *T.* cf. *dunbari* from Baffin Bay (Table 3.2). When comparing the 18S rRNA sequence for *T.* cf. *equalis* (GenBank accession number OR561898) to three specimens of *Parathyasira equalis* from Sweden (Table 3.3) using Blast sequence comparison, we obtained 99.87% similarity. The 18S rRNA sequence of *T.* cf. *dunbari* (GenBank accession number OR561899) was 98.66% similar to the sequences of the three specimens of *P. equalis*. When comparing all three 18S rRNA sequences of *P. equalis* to each other, they matched by 100%. *T.* cf. *dunbari* and *T.* cf. *equalis* 18S rRNA sequences were 98.53% similar to each other.

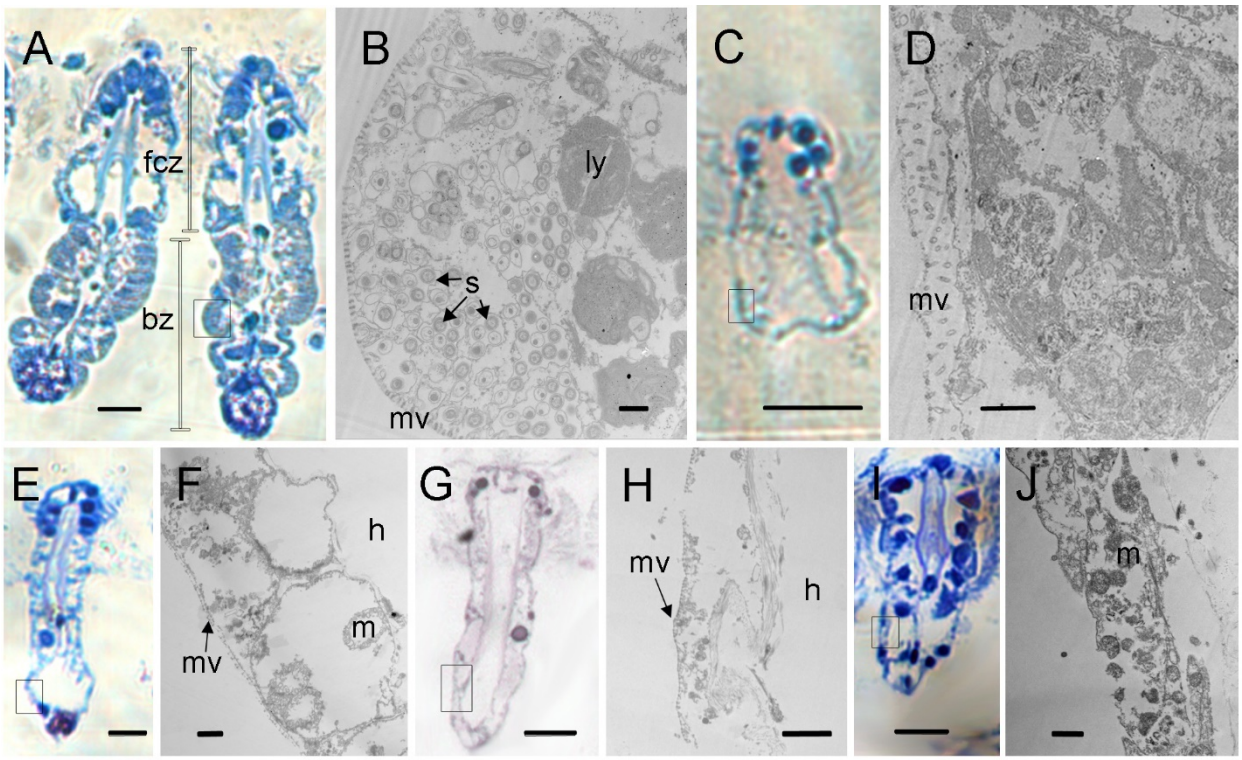

Figure 3.13. Transverse sections of gill filaments of examined thyasirid specimens, viewed through light microscopy (A, C, E, G, I; scale bars = 20 µm) or transmission electron microscopy (B, D, F, J; scale bars = 1 µm; H; scale bar = 2 µm). Gill sections are oriented such that the frontal ciliated zone (fcz) is located at the top of each panel. **A**. *Thyasira* cf. *gouldi*, Frobisher Bay. Abfrontal cells form a bacteriocyte zone (bz), deeply stained with toluidine blue. **B**. Bacteriocyte of *T.* cf. *gouldi* collected in Saglek Bank, corresponding approximately to the area denoted by a rectangle in A. Bacterial symbionts (s) are present between the lysosomes (ly) and microvilli (mv). **C**. *T. plana*, St. Lawrence estuary. **D**. Abfrontal cell of *T. plana*, Frobisher Bay, corresponding approximately the area denoted by a rectangle in C. No symbionts could be seen among microvilli (mv) or intracellularly. **E**. *T.* cf. *equalis*, Baffin Bay, catalog number CMNML 66593. **F**. Abfrontal cell of *T.* cf. *equalis*, representing the rectangular inset in E. The palleal chamber water is on the left of visible epithelia, and hemolymph (h) (at the centre of filament) is on the right. **G**. *T.* cf. *dunbari*, Baffin Bay, catalog number CMNML 66591. **H**. Abfrontal cell of *T. dunbari*, representing the rectangular inset in G. **I**. *Axinopsida orbiculata*, Labrador Sea, catalog number CMNML 43439. **J**. Abfrontal cell of *A. orbiculata*, representing the rectangular inset in G.

28s rRNA

We obtained 547 bp long 28S rRNA sequences of *T*. cf. *equalis* (GenBank accession number OR283032) and *T.* cf. *dunbari* (GenBank accession number OR283033) from Baffin Bay (Table 3.2), and observed 99.45% similarity in the 28S rRNA sequences of *T*. cf. *equalis* and of the three specimens of *P. equalis* in GenBank (Table 3.3). We also observed 98.72% similarity between the three GenBank *P. equalis* sequences and *T. dunbari* from Baffin Bay (Table 3.3), and 98.54% similarity between Baffin Bay *T.* cf. *equalis* and *T.* cf. *dunbari*. *P. equalis* sequences (vouchers BMNH 20070296 and TEQU.SDW.1) showed 99.93% similarity to *P. equalis* (BivAToL-374).

Table 3.2. Species, collection locality and Genbank accession numbers for the genes compared in Table 3.3. Bolded GenBank accession numbers indicate specimens collected in this study. Location unknown for Genbank accessions KC429367 and KC429469.

Species	Locality	18S rRNA	28S rRNA
Thyasira cf. *equalis* 115-1	Baffin Bay (76.3319, -71.2547)	**OR561898**	**OR283032**
Thyasira cf. *dunbari* 111-2	Baffin Bay (76.308, -73.2013)	**OR561899**	**OR283033**
Parathyasira equalis	Sweden, Gullmarsfjord	AM392437	AM392453
Parathyasira equalis	Sweden, Gullmarsfjord	AM779656	AM774482
Parathyasira equalis		KC429367	KC429469

Table 3.3. 18S and 28s (grey cells) rRNA BLAST sequence comparison of *T. equalis* (voucher: 115-1) *T.* cf. *dunbari* (voucher: 111-2) and *Parathyasira equalis* specimens in GenBank. Values indicate percent identity. Query cover is 100% unless indicated otherwise. See Table 3.2 for GenBank accession numbers.

Specimens	*Thyasira* cf. *equalis* Voucher: 115-1	*Thyasira* cf. *dunbari* Voucher: 111-2	*Parathyasira equalis* Voucher: BivAToL-374	*Parathyasira equalis* Voucher: TEQU.SDW.1	*Parathyasira equalis* Voucher: BMNH 20070296
Thyasira cf. *equalis* Voucher: 115-1	100%	98.54%	99.45%	99.45%	99.45%
Thyasira cf. *dunbari* Voucher: 111-2	98.53%	100%	98.72%	98.72%	98.72%
Parathyasira equalis Voucher: BivAToL-374	99.87%	98.66%	100%	99.93%	99.93%
Parathyasira equalis Voucher: TEQU.SDW.1	99.87%	98.66%	100%	100%	100%
Parathyasira equalis Voucher: BMNH 20070296	99.87%	98.66%	100% QC: 99%	100%	100%

3.5 Discussion

3.5.1. Morphology

Shell characters

The shells of Thyasiridae are similar externally: with approximately ovate outline, poorly defined sculptural details, and weakly developed hinge structures (Oliver & Killeen 2002). Identifications rely mainly on differences in shell form along the posterior (most notably the submarginal sulcus, SMS, when present) and anterior shell (lunule), which we described and showed in detail in our study for five thyasirid species (*T.* cf. *gouldi, T. plana*, *T.* cf. *dunbari*, *T. cf. equalis* and *A. orbiculata)* commonly found within Eastern Canada and the Canadian Arctic (Figs. 2.7–2.9, 3.4, 3.5, 3.7, 3.8, 3.10, and 3.11).

We explored differences in shell outline as a potentially unbiased, shape-based tool to distinguish between thyasirids from our study region. Although we observed some differences in outline among the five species, our cluster analysis revealed that these species cannot reliably be discerned based solely on shell outline (Appendix B). The variability of shell outline within each species (most notably for *T.* cf. *equalis*) could explain this result. Similarly, a previous study showed that shell contours, analyzed using the same approach, could not consistently differentiate specimens of *T*. cf. *gouldi* (therein *T*. cf. *gouldi* OTU1 and OTU2) and *T. plana* (therein *T*. cf. *gouldi* OTU3; Batstone *et al.* 2014). To differentiate properly among the five species studied here, researchers must also examine additional shell features, including elements of shell sulci, ferruginous patches and dentition.

T. cf. *gouldi* has a posterior shell with two distinctly rounded folds that give rise to the SMS, a sharp posterior sulcus, and auricle; these characters are more defined in larger shells (Fig. 2.7A, 3.7C–H) and do not occur in the other four species described here. The outline is subequilateral to roundly-subovate with an anterior area expanded laterally. The long, sloping and slightly sunken lunule margin forms a rounded to subacute junction with the anterior margin. The narrowly rounded and laterally angulated anterior-ventral margin forms a continuous rounded curve with the ventral margin (Fig. 2.7), which is similar to the margins observed in larger specimens of *T. plana*. Shells sometimes exhibit small ferruginous patches near the posterior sinus (Fig. 2.7) and the anterior-lunule margin junction (Figs. 2.7 C-E).

Like *T.* cf. *gouldi*, *T. plana* has a subequilateral to subovate outline, but with an anterior area expanded only slightly laterally (Fig. 2.9). The moderately long, slightly sloping, and not sunken lunule margin forms a rounded junction with the anterior margin. The continuous rounded curve of the anterior-ventral margin only angulates slightly laterally in larger shells. The posterior area defines the main difference when comparing to *T.* cf. *gouldi*; a weakly binsinuated posterior margin and a shallow sulcus characterizes *T. plana*, though absent in some specimens (Figs. 2.9D, I, J), along with a shallow SMS. Ferruginous patches can occur near the posterior sinus (Figs. 2.9C, G), sometimes expanded laterally along the posterior area (Figs. 2.9I, J), and at the anterior-lunule margin junction (Figs. 2.9A, G, I, J).

T. cf. *dunbari* has an elongated outline, expanded antero-ventrally in larger adult specimens (Figs. 3.7A, B), but equilateral-subovate in smaller specimens (Figs. 3.7E, F). The long, flat posterior area defines this species, with an angular to narrowly rounded margin, and a sharp and well defined SMS (Figs. 3.7G–I). This species can be confused with *T. equalis*

(Ockelmann 1958; Oliver & Killeen 2002) and *T.* cf. *equalis*: the shape of the posterior margin of *T. dunbari* can vary from slightly angular (Figs. 3.7A–C, E) to sharply angular (Figs. 3.7D, F), depending on specimen size. Other features, such as the spoon shaped callus mentioned in Lubinsky (1976) may not be a useful character in identifying this species, noting that it was not visible in specimens examined in our study. The specimen shown in Lubinsky (1976) is large (referred to as an adult, Plate. Fig. 5 of Lubinski, 1976) in comparison to our presumably younger specimens (length ranging from 2.39–4.72 mm, Table 3.1), which could explain the absence of this feature in our assessment. Lubinsky (1976) did not specify whether a spoon shaped callus was present on both adult and young specimens.

T. cf. *equalis* has a unique posterior area that varied greatly among the specimens we examined, with a sharply truncated margin, either straight (Fig. 3.4G) or slightly curved (Figs. 3.4A, B, D). One weakly rounded fold and a long, deep, and sharply defined SMS characterize the posterior area (Fig. 3.4C). The outline is equilateral to ovate-polygonal shaped. Two measured specimens of *T. equalis* sensu stricto (cmnml06144 from Isle of Shoals, N.H., USA) (prodissoconch: 139–164 μm; shell length: 2.87–5 mm, Appendix A) are similar in shell length only when compared to *T.* cf. *equalis* (prodissoconch: 125–148 μm; shell length: 2.42–4.94 mm, Table 3.1). The prodissoconch measurements indicate that *T. equalis* s.s does not follow a longitudinal trend (Oliver and Killeen 2002). As stated above, this species has been mistaken for *T.* cf. *dunbari* (Ockelmann 1958; Oliver & Killeen 2002) as a result of the variation in the posterior margin, which contributes to our suspicion of cryptic diversity within *T. equalis* (see also 3.5.3, below).

The circular (Figs. 3.10A–D) to subcircular outline (Figs. 3.10E–G) and deeply sunken anterior dorsal margin in *A. orbiculata* represents a key identification character for this species. The posterior dorsal margin is gently curved, while the ventral margin is broadly rounded. It has a distinct lunule (Oliver & Killeen 2002) (Fig. 3.10) and the flat posterior area lacks sulci. This species has a distinctly large cardinal tooth on the right valve hinge (Figs. 3.11D, E), with a corresponding socket on the left valve hinge, which must be confirmed when identifying *A. orbiculata*.

The larval shell (prodissoconch) size can be a valuable character in separating thyasirid species (see Table 3.1) when used in combination with other identification characters. Size ranges show some overlap between 1) *T.* cf. *gouldi*: 155–238 µm and *T. plana*: 130–219 µm; 2) *T.* cf. *equalis*: 125–148 µm and *T.* cf. *dunbari*: 119–179 µm, though *A. orbiculata* has a uniquely large prodissoconch of 260–338 µm. Part of these overlaps could reflect the latitudinal trend observed in thyasirids, where prodissoconchs increase in size from south to north (Ockelmann, pers. comm., in Oliver & Killeen 2002; Chapter 2). This feature is very useful in identifying *A. orbiculata*.

Internal anatomy

Internally, some species within the Thyasiridae have a modified anatomy that supports chemosymbiosis, with enlarged gills that house sulphur oxidising bacteria (Allen, 1958; Dando & Southward, 1986; Southward 1986; Dufour 2005). Symbiont presence can be determined through examination of thin sections of gill filaments (Figure 3.13). Of the five species examined, only *T.* cf. *gouldi* has symbionts (Figure 3.13); its elongated type 3 gill structure (Dufour 2005) was described previously in Bonne Bay specimens (Batstone et al. 2014) and for

Saglek Bank and Frobisher Bay specimens (Chapter 2). *T. plana*, *T.* cf. *equalis, T.* cf. *dunbari,* and *A. orbiculata* all lack symbionts, and correspondingly, their gill structure shows no apparent expansion of the abfrontal area (gill type 2 of Dufour 2005; Figs. 3.13E, F). These results agree with the published findings in Dufour (2005) for *T. equalis* from Raunefjord, Norway (N=71), Barents Sea (N=2), and North Sea (N=30) and *A. orbiculata* from Tatar Strait (N=2) and East Greenland (N=3). Here, we present the first documentation of gill structure for *T.* cf. *dunbari*, while confirming the absence of symbionts for this species. These observations provide some of the first direct evidence for symbiont presence or absence in Arctic thyasirids, which have received little attention to date.

Similar anatomical characters across the five species include: 1) adductor scars unequal in size with the anterior adductor being larger and elongated; 2) vermiform foot with a bulbous tip (less apparent in *A. orbiculata*); 3) ctenidia (gills) consisting of both inner and outer demibranchs; 4) divided and lobed lateral body pouch; 5) mantle fused posteriorly to form an exhalent opening beneath the posterior adductor, but no fusion in the ventral and anterior margins (Fig. 2.8; Figs. 3.5, 3.8, and 3.11). *T.* cf. *gouldi* has large, thick, and fleshy gills compared to the other species, which corresponds to the presence of symbionts (Fig. 2.8B). Across species examined, the foot is typically long with a swollen distal end, though in *A. orbiculata* we observed a less vermiform, unswollen tip, and shorter foot (Fig. 3.11C), which suggests it may not extend as deeply in sediments as the other species (Payne & Allen 1991). The lateral body pouch is distinctly large, branched, and lobed in *T.* cf. *gouldi*, *T.* cf. *dunbari*, and *T.* cf. *equalis* (Fig. 2.8B; 3.5A-D and 3.8). The lateral body pouch is smaller, slightly branched, and less lobed in *T. plana* and *A. orbiculata* (Fig 2.8D, 3.11A–C). In *T. dunbari*, we

observed that the lateral body pouch can be a dark brown to black in color when fresh (Fig. 3.8C, E), but this pigmentation is lost when preserved in ethanol. Anatomic features are an important and useful addition to the identification of thyasirid species.

3.5.2 The generic assignment of thyasirid species

The genus *Parathyasira* is currently designated as an accepted taxon (WoRMS Editorial Board 2022) that includes 14 species, two of which are *P. equalis* and *P. dunbari*. The type taxon is *Parathyasira resupina* Iredale 1930, and the features considered diagnostic for the genus are: 1) posterior shell flattened with a defined submarginal sulcus surrounding the ligament, auricle absent; 2) posterior margin angled and truncated with no sinuation; 3) outline relatively equilateral, ovate to ovate-rhomboidal with an obvious beak (Oliver & Killeen 2002). Over time, researchers have assigned some species interchangeably to the genus *Thyasira*, the genus *Parathyasira*, or to a subgenus *Parathyasira* within the genus *Thyasira*; WoRMS does not currently accept the subgeneric status. Payne & Allen (1991) placed *Thyasira* species (most notably *T. equalis* and *T. dunbari*) into *Parathyasira*, which other authors follow (Oliver & Killeen 2002; Zelaya 2009; Huber 2015). Subsequent studies have placed species in or out of *Parathyasira*, for example: 1) Zelaya (2009) placed *Thyasira dearborni* (Nicol 1965) in *Parathyasira*; and 2) Huber (2015) transferred three species of *Parathyasira* (*Parathyasira granulosa* (Monterosato, 1874), *Parathyasira kaireiae* (Okutani *et al*., 1999), and *Parathyasira subcircularis* (Payne & Allen, 1991) into the genus *Thyasira*. Oliver (2015) and Kamenev (2020) disagree with Huber (2015) and argue for retaining these species within the genus *Parathyasira*, illustrating the confusion and inconsistencies with generic and subgeneric definitions within the Thyasiridae. As mentioned in Oliver (2015), the type species of *Parathyasira* (*P. resupina*) has a

spiny micro-sculpture arranged in radial rows of short spines, a character not observed in many species assigned to *Parathyasira*. Oliver (2015) limited the diagnosis of the genus *Parathyasira* only to species with a similar shell micro-sculpture; notably, the genus *Parathyasira* still comprises both smooth and radially sculptured species in WoRMS. Furthermore, anatomical details of the type species of *Parathyasira* are unknown. We agree with Oliver & Rodrigues (2017) and Kamenev (2020) in that this ambiguity illustrates the current unsatisfactory generic definitions within the Thyasiridae. Given the incomplete definition of *Parathyasira*, we use the original genus of *Thyasira* for *T. equalis* and *T. dunbari* in this study, unless referring to genetic data. Based on our results, we expect that the taxon referred to as *T. equalis* may well encompass multiple species.

3.5.3 Possible cryptic diversity within *T. equalis*

As a result of: 1) considerable variations in shell outline reported here and elsewhere (Oliver & Killeen 2002); 2) considerable differences in genetic sequences (Table 3.2); 3) specimens frequently confused with *T.* cf. *dunbari* and numerous synonymies erroneously inferred (Oliver & Killeen 2002), we suspect cryptic diversity exists within *Thyasira equalis* as currently defined. The shell outline along the posterior margin differs markedly among specimens; although always indented, the shape varies from a sharp straight edge (Fig. 3.4E–G) to small protruding curves (Fig. 3.4A, B, D). The proper identification of *T. equalis* will require more information, such as suitable material from the type location for genetic sequencing and internal anatomy examination for comparison with *T. equalis* specimens found elsewhere.

Here we provide new sequences for *T.* cf. *equalis* (Table 3.3) and based on this data, we show evidence that European *T. equalis* specimens differ slightly from our newly studied specimens. This discovery calls for additional research on the European *T. equalis* and the need for further work to resolve species resembling *T. equalis* on a larger geographic scale.

3.5.4 Expanded distribution

Here, we provide new documented distributions for *T.* cf. *gouldi* (Fig. 3.2), *T. plana* (Fig. 3.3), *T.* cf. *equalis* (Fig. 3.6), *T.* cf. *dunbari* (Fig. 3.9), and *A. orbiculata* (Fig. 3.12) based on specimens examined; see Appendix A for exact coordinates. Locations and depths observed correspond to previous reports for all species (excluding the locations for *T.* cf. *dunbari* (Fig. 3.9) (Oliver & Killeen 2002; CaRMS Editorial Board 2022; Verrill & Bush 1898; Lubinsky 1976; Table 2.1; Appendix A).

Following the descriptions of *T. cf. gouldi* and *T. plana* in Chapter 2, we herein provide an expanded distribution in Arctic waters for these species after examining and confirming species identification in specimens from the CMN and *Amundsen* cruise in July 2017 (Figs. 3.2, 3.3, respectively). Depths, as recorded on museum collections, fall within a similar range as previously stated for both species: 1) *T.* cf. *gouldi*: 1–380 m; 2) *T. plana*: 1–610 m.

Specimens identified as *T.* cf. *equalis* from the CMN and Baffin Bay were found at depths of 15–610 m, which falls within the same range as *T. equalis* described by Kurt Ockelmann in Oliver & Killeen (2002) of 10–2700 m. The specimens examined within this study show a subset of the broader range in which this species is found, most notably in arctic waters.

Our study indicates that *T.* cf. *dunbari* is found further south than *T. dunbari*. Prodissoconch measurements seen in Table 3.1 show that *T.* cf. *dunbari* larval shell increases in size from south to north, a trend also observed in *T.* cf. *gouldi* and *T. plana* (Chapter 2). *T. dunbari* depths range from 10–70 m in the NW region of the Canadian archipelago north from the M'Clure Strait-Lancaster Sound (Lubinsky, 1976) to <100 m in Kongsfjord, Svalbard (Åström *et al.*, 2018), corresponding with the shallow (30 to 40 m) inner glacial bay depths of *T.* cf. *dunbari* in Bonne Bay, NL. *T.* cf. *dunbari* specimens from the CMN occur at greater depths, upwards to 931 m in Arctic waters at latitudes >75˚. *T. dunbari* (or possibly *T*. cf. *dunbari*) was described as cold water, stenothermal species can tolerate temperature submergence at great depth just NE of the Faroes (Oliver & Killeen 2002). *T.* cf. *dunbari* seems to be a highly adaptive species based on its broad habitat range (Åström *et al.*, 2018).

Past studies documented *A. orbiculata* as an Arctic species ranging into Iceland (Madsen 1949), commonly in shallow subtidal glacial sediments of fjords in depths up to a few hundred metres (Aitken & Fournier 1993; Włodarska-Kowalczuk 2007). In this study, we identified specimens from the Foxe Basin and Frobisher Bay in Arctic waters of similar depths (0–28 m) and environments. We could not determine the depth of specimens from the CMN off Greenland.

3.6 Conclusions

Our study revised the species assignment of many specimens at the CMN, highlighting poor understanding of the identification of thyasirids in the Canadian Arctic and Eastern Canada. Based on our analysis, some gaps remain in understanding the classification of the thyasirid species occurring in these regions and more broadly. The overview provided here improves the identification of species, while bringing attention to taxa that require further examination. We

present new gene sequences for *T.* cf. *equalis*, documenting the species in Arctic waters, but emphasize the need for further taxonomic work on the possibly cryptic species *T. equalis*. Future work of cryptic species complexes require attention, including those that occur in Western Canada (e.g., British Columbia *Thyasiria gouldi* complex), which we did not include in our research. We emphasize the importance of prodissoconch measurements and soft tissue examination as key characters in identifying thyasirid species. We provide some of the first evidence for symbiont presence or absence in Arctic thyasirids and suggest giving this character more attention in future studies because it can help define species identifications and their role in the environment. We also provide the first documentation of gill structure, gene sequence and distribution of *T.* cf. *dunbari*.

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Chapter 4: Summary and Conclusions

4.1 Summary

This study sought to address and understand better the taxonomic challenges within the identifications of thyairid species found within Eastern Canada and the Canadian Arctic. In Chapter 2, we investigated the *Thyasira gouldi* complex (described in Batstone *et al*. 2014) through an analysis of the anatomical, genetic, and symbiotic characters of thyasirids resembling *T. gouldi* from Bonne Bay, NL and two Canadian Arctic locations: Saglek Bank and Frobisher Bay. We designated the asymbiotic members of these thyasirids to a species previously synonymized with *T. gouldi*: *T. plana*, while retaining the designation *T.* cf. *gouldi* for symbiotic members retain until specimens from the type location (*T. gouldi* s.s) become available and more information, such as soft anatomy and gene sequences can be examined. In Chapter 3, we highlight the poor understanding of the identification of thyasirids in the Canadian Arctic and Eastern Canada. We described new information for thyasirid taxa (*Thyasira* cf. *gouldi*, *Thyasira plana*, *T.* cf. *dunbari*, *T.* cf. *equalis* and *Axinopsida orbiculata*) commonly found in these regions, which include: measurements, internal and external morphological characters, new gene sequences for *T.* cf. *equalis* and *T.* cf. *dunbari,* distributions of examined specimens, and descriptions outlining key identifying characters. We emphasize the need for further taxonomic work on the possibly cryptic species *T. equalis*.

4.2 Conclusions

In Chapter 2, we identify both *T. plana* and *T.* cf. *gouldi* from fjords and a possible seep, where each environment has sediments of high organic content. *T. plana* and *T.* cf. *gouldi* slightly differ from each other in shell outline and in the location of a ferruginous patch on the dorsal end of the shell, in the degree of development of gill filaments, and in the presence or absence of symbionts. We document the presence of bacterial symbionts for the first time in thyasirids (*T.* cf. *gouldi*) from the Canadian Arctic. *T.* cf. *gouldi* and *T. plana* have a smaller prodissoconch than *T. gouldi* s.s., and their prodissoconch increases in size with increasing latitude.

Based on our analysis in Chapter 3, some gaps remain in understanding the classification of the thyasirid species in the Canadian Arctic and Eastern Canada and more broadly. We present new gene sequences for *T. cf. equalis*, documenting the species in Arctic waters. We provide some of the first evidence for symbiont presence or absence in Arctic thyasirids. We also provide the first documentation of gill structure, gene sequence and a distribution for *T. cf. dunbari*.

4.3 Future directions

This work suggests that the proper delineation of species within the *T. gouldi* complex (those with and without symbionts) requires a careful and further examination of multiple morphological and genetic characters across a range of geographical locations, including specimens (*T. gouldi* s.s) from the type location (Massachusetts). We designated symbiotic specimens from Bonne Bay and Arctic locations as *T.* cf. *gouldi*, and further studies may indicate

that they form a separate species from *T. gouldi* s.s. For the proper diagnosis of thyasirids, we highlight the importance of examining the larval shell size, while considering latitude.

The overview provided within Chapter 3 improves the identification of species, while bringing attention to others that require further examination (most notably the suspected cryptic species complex of *T. equalis*). Future work on cryptic species complexes require attention, including those in Western Canada (e.g., British Columbia *Thyasiria gouldi* complex), which we did not consider. We emphasize the importance of prodissoconch measurements and soft tissue examination as key characters in identifying thyasirid species. Finally, we suggest a need to give symbiont presence or absence in thyasirids more attention in future studies, as it can help define species identifications and their role in the environment.

4.4 Reference

Batstone, R. T., J. R. Laurich, F. Salvo, and S. C. Dufour. (2014). Divergent chemosymbiosis-related characters in *Thyasira* cf. *gouldi* (Bivalvia: Thyasiridae). *PLoS ONE*. 9: e92856.

Appendix A. List of specimens examined, with associated metadata.

Coordinates:

The red coordinates are estimated through location description for map. The coordinate information was missing or not provided on specimen label during the Canadian Museum of Nature data collection.

NA or blank cells:

This reflects specimen information is unknown, lost, or was not collected during sampling.

Specimen ID	Museum vouchers	Original Identification	Revised Identification	Species name	OTU	Lat. (DD)	Long. (DD)	Location	Vessel	Station	Depth (m)	Date sampled	Prodissoconch (µm)	Length (mm)	Collector
St. 323-1	na	*Thyasira dunbari*	na	*Thyasira dunbari*	na	74.1566	-80.4679	Baffin Bay	CCGS Amundsen	na	787	2017-07-31	na	2.944	Bárbara Neves
St. 111-1	na	*Thyasira dunbari*	na	*Thyasira dunbari*	na	76.3080	-73.2013	Baffin Bay	CCGS Amundsen	na	na	2017-07-29	152	na	Bárbara Neves
St. 111-2	na	*Thyasira dunbari*	na	*Thyasira dunbari*	na	76 18.486	73 12.428	Baffin Bay	CCGS Amundsen	na	593	2017-07-29	na	na	Bárbara Neves
St. 111-3	na	*Thyasira equalis*	na	*Thyasira equalis*	na	76.3081	-73.2071	Baffin Bay	CCGS Amundsen	na	593	2017-07-29	130	2.784	Bárbara Neves
St. 111-4	na	*Thyasira equalis*	na	*Thyasira equalis*	na	76.3081	-73.2071	Baffin Bay	CCGS Amundsen	na	593	2017-07-29	142	2.634	Bárbara Neves
St. 323-2	na	*Thyasira equalis*	na	*Thyasira equalis*	na	74.1566	-80.4679	Baffin Bay	CCGS Amundsen	na	787	2017-07-31	128	2.273	Bárbara Neves
St. 115-1	na	*Thyasira equalis*	na	*Thyasira equalis*	na	76.3319	-71.2547	Baffin Bay	CCGS Amundsen	na	668	2017-07-26	125	2.375	Bárbara Neves
cmnml43439	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	64.0681	-51.6276	Godthaab off Lille Malene, Greenland	na	na	na	1905-05-09	321	4.671	na
cmnml43439	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	64.0681	-51.6276	Godthaab off Lille Malene, Greenland	na	na	na	1905-05-09	338	3.971	na
cmnml43439	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	64.0681	-51.6276	Godthaab off Lille Malene, Greenland	na	na	na	1905-05-09	267	3.393	na
cmnml43439	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	64.0681	-51.6276	Godthaab off Lille Malene, Greenland	na	na	na	1905-05-09	293	2.843	na
cmnml43439	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	64.0681	-51.6276	Godthaab off Lille Malene, Greenland	na	na	na	1905-05-09	321	4.375	na
cmnml42463	na	*Thyasira dunbari*	*Axinopsida orbiculata*	*Axinopsida orbiculata*	na	63.6410	-68.6210	NWT: Frobisher Bay	na	na	na	na	na	4.482	DV Ellis
cmnml45579	na	*Thyasira gouldi*	*Axinopsida orbiculata*	*Axinopsida orbiculata*	na	69.1000	-79.1830	Foxe Basin	na	Stn. 901	0-9	1957-07-22	272	2.411	FBR "Calanus"
cmnml45579	na	*Thyasira gouldi*	*Axinopsida orbiculata*	*Axinopsida orbiculata*	na	69.1000	-79.1830	Foxe Basin	na	Stn. 901	0-9	1957-07-22	260	na	na
cmnml45579	na	*Thyasira gouldi*	*Axinopsida orbiculata*	*Axinopsida orbiculata*	na	69.1000	-79.1830	Foxe Basin	na	Stn. 901	0-9	1957-07-22	301	2.763	na
cmnml45579	na	*Thyasira gouldi*	*Axinopsida orbiculata*	*Axinopsida orbiculata*	na	69.1000	-79.1830	Foxe Basin	na	Stn. 901	0-9	1957-07-22	na	2.775	na
cmnml70476	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	49.5029	-57.8201	East Arm Bonne Bay, Newfoundland	na	Acc. 71-184	226	1971-07-25	141	3.791	Ambrose Foote
cmcnml73960	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	48.3083	-69.1267	St. Lawrence R., Estuary, P.Q.	na	stn. B230, Acc. 1974-62	110	1971-07-07	162	4.724	G. Robert
cmcnml73960	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	48.3083	-69.1267	St. Lawrence R., Estuary, P.Q.	na	stn. B230, Acc. 1974-62	110	1971-07-07	132	3.674	G. Robert
cmcnml73960	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	48.3083	-69.1267	St. Lawrence R., Estuary, P.Q.	na	stn. B230, Acc. 1974-62	110	1971-07-07	145	3.896	G. Robert
cmnml2018-2482	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	53.8000	-78.9583	Quebec, La Grande River	na	300, Sn. 62, Ind. 7, Acq: 1	7	1979-09-07	149	3.654	FBR., Arctic Biological Station
cmnml45343	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	56.1849	-76.3642	NE of Cairn Island, Richmond Gulf	na	na	30-40	1963-07-21	122	na	AHC 508
cmnml66587	na	*Thyasira equalis*	*Thyasira dunbari*	*Thyasira dunbari*	na	73.3233	-73.0417	Baffin Bay	na	Stn. 1033, Acc. 70-205	931	1970-09-09	150	3.112	AHC & BK
cmnml66587	na	*Thyasira equalis*	*Thyasira dunbari*	*Thyasira dunbari*	na	73.3233	-73.0417	Baffin Bay	na	Stn. 1033, Acc. 70-205	931	1970-09-09	123	3.118	AHC & BK
cmnml66587	na	*Thyasira equalis*	*Thyasira dunbari*	*Thyasira dunbari*	na	73.3233	-73.0417	Baffin Bay	na	Stn. 1033, Acc. 70-205	931	1970-09-09	123	2.718	AHC & BK
cmnml66587	na	*Thyasira equalis*	*Thyasira dunbari*	*Thyasira dunbari*	na	73.3233	-73.0417	Baffin Bay	na	Stn. 1033, Acc. 70-205	931	1970-09-09	128	2.823	AHC & BK
cmnml66591	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	66.1750	-57.5600	On saddle of Cape Walsingham-Holsteinborg Ridge	na	Stn. 1039, Acc. 70-205	574	1970-09-14	146	2.989	AHC & BTK
cmnml66591	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	66.1750	-57.5600	On saddle of Cape Walsingham-Holsteinborg Ridge	na	Stn. 1039, Acc. 70-205	574	1970-09-14	141	2.909	na
cmnml66591	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	66.1750	-57.5600	On saddle of Cape Walsingham-Holsteinborg Ridge	na	Stn. 1039, Acc. 70-205	574	1970-09-14	136	2.391	na
cmnml66591	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	66.1750	-57.5600	On saddle of Cape Walsingham-Holsteinborg Ridge	na	Stn. 1039, Acc. 70-205	574	1970-09-14	119	2.425	na
cmnml79395	na	*Thyasira dunbari*	na	*Thyasira dunbari*	na	82.5069	-62.2853	Ellesmere Island, Alert, Parr Inlet, NWT	na	SM 10	na	1954-Jul-Aug	na	na	SD MacDonald
cmnml66593	na	*Thyasira gouldi*	*Thyasira dunbari*	*Thyasira dunbari*	na	76.0133	-71.3233	Baffin Bay	na	na	475	1971-09-20	179	3.416	na
Arctique 2010	na	*Thyasira* sp.	*Thyasira dunbari*	*Thyasira dunbari*	na	74.1887	-83.9737	Lancastor Sound	na	301	671	2010-08-07	na	na	Virginie Roy
Arctique 2011	na	*Thyasira* sp.	*Thyasira dunbari*	*Thyasira dunbari*	na	68.9995	-106.5608	Dease Strait	na	314_3b	122	2011-10-06	na	na	Virginie Roy
Arctique 2010	na	*Thyasira* sp.	*Thyasira equalis*	*Thyasira equalis*	na	74.1887	-83.9737	Lancastor Sound	na	301	671	2010-08-07	na	na	Virginie Roy
Arctique 2011	na	*Thyasira* sp.	*Thyasira equalis*	*Thyasira equalis*	na	68.9995	-106.5608	Dease Strait	na	314_3b	122	2011-10-06	na	na	Virginie Roy
cmnml66587	na	*Thyasira equalis*	na	*Thyasira equalis*	na	73.3233	-73.0417	Baffin Bay	na	Stn. 1033, Acc. 70-205	931	1970-09-09	144	2.461	AHC & BK
cmnml66587	na	*Thyasira equalis*	na	*Thyasira equalis*	na	73.3233	-73.0417	Baffin Bay	na	Stn. 1033, Acc. 70-205	931	1970-09-09	131	2.878	AHC & BK
cmnml45295	na	*Thyasira gouldi*	*Thyasira equalis*	*Thyasira equalis*	na	57.3500	-76.7956	Nastapoka Sound, off Broughlou Island, NWT	na	na	91	1963-07-16	142	4.941	AHC 483
cmnml66591	na	*Thyasira gouldi*	*Thyasira equalis*	*Thyasira equalis*	na	66.1750	-57.5600	on saddle of Cape Walsingham-Holsteinborg Ridge	na	Stn. 1039, Acc. 70-205	574	1970-09-14	148	2.290	na
cmnml66591	na	*Thyasira gouldi*	*Thyasira equalis*	*Thyasira equalis*	na	66.1750	-57.5600	on saddle of Cape Walsingham-Holsteinborg Ridge	na	Stn. 1039, Acc. 70-205	574	1970-09-14	134	2.425	na
cmnml66593	na	*Thyasira gouldi*	*Thyasira equalis*	*Thyasira equalis*	na	76.0133	-71.3233	Baffin Bay	na	Stn. 1057, Acc. 71-246	475	1971-09-20	na	3.415	na
cmnml06144	na	*Thyasira gouldi*	*Thyasira equalis*	*Thyasira equalis*	na	42.9897	-70.6082	Isle of Shoals, N.H., U.S.A.	na	na	99	1953-04-09	139	2.869	AH Clarke
cmnml06144	na	*Thyasira gouldi*	*Thyasira equalis*	*Thyasira equalis*	na	42.9897	-70.6082	Isle of Shoals, N.H., U.S.A.	na	na	99	1953-04-09	164	3.411	AH Clarke
cmnml36479	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.0500	-80.7333	Hudson Bay	na	na	73	1959-09-01	196	na	na
cmnml36479	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.0500	-80.7333	Hudson Bay	na	na	73	1959-09-01	227	na	na
cmnml41792	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	59.9000	-69.5167	Ungawa Bay	na	na	64	1947-07-18	209	na	FBR "Calanus"
cmnml35052	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.2305	-78.9552	South of Spence Harbour, Hudson Bay	na	na	na	1958-07-05	201	na	FBR "Calanus"
cmnml35052	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.2305	-78.9552	South of Spence Harbour, Hudson Bay	na	na	na	1958-07-05	199	na	FBR "Calanus"
cmnml35052	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.2305	-78.9552	South of Spence Harbour, Hudson Bay	na	na	na	1958-07-05	216	na	FBR "Calanus"
cmnml35202	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.2305	-78.9552	Baffin Island, SE of Sleeper Island N.W.T.	na	na	90-95	1958-09-16	221	na	FBR "Calanus"
cmnml35202	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.2305	-78.9552	Baffin Island, SE of Sleeper Island N.W.T.	na	na	90-95	1958-09-16	189	na	FBR "Calanus"
cmnml35202	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.2305	-78.9552	Baffin Island, SE of Sleeper Island N.W.T.	na	na	90-95	1958-09-16	199	na	FBR "Calanus"
cmnml35079	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	55.9887	-78.9458	Hudson Bay, N. Omarolluk Sound	na	na	na	1958-Jul	221	na	FBR "Calanus"
cmnml35079	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	55.9887	-78.9458	Hudson Bay, N. Omarolluk Sound	na	na	na	1958-Jul	222	na	FBR "Calanus"
cmnml41768	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	69.1000	-79.1833	Foxe Basin	na	na	3-34	1905-05-10	218	na	FBR "Calanus"
cmnml35177	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	59.0667	-60.4500	btw Omowa Island and Quebec coast, Hudson Bay	na	na	113	1958-09-10	202	na	FBR "Calanus"
cmnml35177	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	59.0667	-60.4500	btw Omowa Island and Quebec coast, Hudson Bay	na	na	113	1958-09-10	217	na	FBR "Calanus"
cmnml45343	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.1849	-76.3642	NE of Cairn Island, Richmond Gulf	na	na	30-40	1963-07-21	na	na	AHC 508
cmnml36383	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	69.1375	-78.9081	Frustration Bay, Rowley Island	na	na	na	1957-07-05	na	na	FBR "Calanus"
cmnml36467	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	52.2833	-78.8333	James Bay, N.W.T	na	na	na	1959-06-30	210	na	FBR "Calanus"
cmnml36467	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	52.2833	-78.8333	James Bay, N.W.T	na	na	na	1959-06-30	na	na	FBR "Calanus"
cmnml41335	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.8153	-76.8361	Nastapoka Island	na	na	na	1905-05-16	216	na	AHC
cmnml41335	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.8153	-76.8361	Nastapoka Island	na	na	na	1905-05-16	217	na	AHC
cmnml35027	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.4417	-78.7917	N. Omarolluk Sound, Hudson Bay	na	na	40	1958-06-27	210	na	Calarius
cmnml27386	na	*Thyasira*	*Thyasira plana*	*Thyasira plana*	na	57.0000	-76.6667	1 mile west of mouth of Nastopoka River, Hudson Bay	na	na	47-53	1963-07-17	na	na	AHC

cmnml27723	na	*Thyasira*	*Thyasira plana*	*Thyasira plana*	na	47.6654	-65.6313	Off youghall, NB.	na	na	42	1911-09-20	na	na	LHY
cmnml30695	na	*Thyasira*	*Thyasira plana*	*Thyasira plana*	na	56.9082	-76.5541	1 mile S.W. of mouth of Nastopoka R., Nastopoka Sd., Hudson Bay, N.W.T.	na	na	na	1963-07-17	na	na	AHC
cmnml41258	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.2501	-76.3320	1/3 of way from head to mouth of small bay just S Gulf Hazard SW of Richmond Gulf P.Q	na	na	na	1963-07-22	na	na	FBR "Calanus"
cmnml41258	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	56.2501	-76.3320	1/3 of way from head to mouth of small bay just S Gulf Hazard SW of Richmond Gulf P.Q	na	na	na	1963-07-23	na	na	FBR "Calanus"
cmnml41738	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	69.6200	-81.1000	Futy & Hecla Strait Foxe Basin	na	na	106	1956-08-17	na	na	FBR "Calanus"
cmnml45338	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	52.2405	-78.5456	Eastmain R., 8 mi E of Eastmain Que.	na	na	4-5	1963-07-04	na	na	AHC
cmnml45580	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	69.3417	-81.7250	Foxe Basin, Hooper Inlet	na	na	51	1956-09-02	na	na	FBR "Calanus"
cmnml45580	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	69.3417	-81.7250	Foxe Basin, Hooper Inlet	na	na	51	1956-09-02	na	na	FBR "Calanus"
cmnml2018-3158	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	69.3085	-106.6016	Arctic Ocean, Starvation Cove	na	na	4-90	1967-08-19	na	na	FBR., Arctic Biological Station
cmnml2018-2482	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	53.8000	-78.9583	Quebec, La Grande River	na	na	7	1979-09-07	na	na	FBR., Arctic Biological Station
cmnml2018-2484	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	53.8000	-78.9583	Quebec, La Grande River	na	na	na	1974-09-07	na	na	FBR., Arctic Biological Station
cmnml2018-2485	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	52.2419	-78.5333	Quebec, Eastmain River	na	na	1.2-1.5	1974-10-02	na	na	FBR., Arctic Biological Station
cmnml2018-2486	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	52.2419	-78.5333	Quebec, Eastmain River	na	na	0.9-1.8	1974-09-19	na	na	FBR., Arctic Biological Station
cmnml70596	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	72.7500	-94.1283	Creswell Bay	na	na	na	1962-06-29	199	na	FRB
cmnml66593	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	76.0133	-71.3233	Baffin Bay	na	na	475	1971-09-20	na	4.161	na
cmnml66593	na	*Thyasira gouldi*	*Thyasira plana*	*Thyasira plana*	na	76.0133	-71.3233	Baffin Bay	na	na	475	1971-09-20	194	3.038	na
cmnml00209	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	54.2756	-55.9573	Labrador	na	na	na	1905-02-06	213	na	na
cmnml00209	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	54.2756	-55.9573	Labrador	na	na	na	1905-02-06	223	na	na
cmnml00209	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	54.2756	-55.9573	Labrador	na	na	na	1905-02-06	197	na	na
cmnml36479	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	56.0500	-80.7333	Hudson Bay	na	na	73	1959-Sep	190	na	na
cmnml45578	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	60.3917	-64.8667	Forbes Sound, Ungawa Bay	na	na	90-130	1949-06-30	212	na	FBR "Calanus"
cmnml36383	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	69.1375	-78.9081	Frustration Bay, Rowley Island	na	na	na	1957-07-05	192	na	FBR "Calanus"
cmnml27346	na	*Thyasira*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	47.6631	-65.6326	19 miles from Youghall, NB.	na	na	na	1911-10-21	na	na	CHY
cmnml41758	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	69.1000	-79.1833	Foxe Basin	na	na	3-34	1957-06-08	na	na	FBR "Calanus"
cmnml41812	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	63.6000	-82.0000	Evanstrait, Hudson Bay	na	na	73-75	1953-09-04	na	na	FBR "Calanus"
cmnml66595	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	75.8450	-74.2900	Baffin Bay	na	na	380	1971-09-26	na	na	AHC
cmnml41738	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	69.6200	-81.1000	Futy & Hecla Strait Foxe Basin	na	na	106	1956-08-17	na	na	FBR "Calanus"
cmnml45580	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	69.3417	-81.7250	Foxe Basin, Hooper Inlet	na	na	51	1956-09-02	na	na	FBR "Calanus"
cmnml2018-2482	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	53.8000	-78.9583	Quebec, La Grande River	na	na	7	1979-09-07	na	na	FBR., Arctic Biological Station
cmnml2018-2482	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	53.8000	-78.9583	Quebec, La Grande River	na	na	7	1979-09-07	na	na	FBR., Arctic Biological Station
cmnml2018-2482	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	53.8000	-78.9583	Quebec, La Grande River	na	na	7	1979-09-07	na	na	FBR., Arctic Biological Station
cmnml2018-2482	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	53.8000	-78.9583	Quebec, La Grande River	na	na	7	1979-09-07	na	na	FBR., Arctic Biological Station
cmnml2018-2487	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	52.2419	-78.5333	Quebec, Eastmain River	na	na	0.9	1974-09-19	na	na	FBR., Arctic Biological Station
cmnml2018-2488	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	53.2419	-79.5333	Quebec, Eastmain River	na	na	0.9-1.8	1974-09-19	na	na	FBR., Arctic Biological Station
cmnml42098	na	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	*Thyasira* cf. *gouldi*	na	41.3800	-63.7500	Georges Bank	na	na		1905-03-26	220	na	W. F. Clapp
cmnml01302	na	*Thyasira gouldi*	na	*Thyasira gouldi*	na	na	na	Ship Channel, Vancouver Island, BC	na	na		May-Aug 1909	173	na	Young & Spreadborough
cmnml01138	na	*Thyasira gouldi*	na	*Thyasira gouldi*	na	na	na	Port Simpson, BC	na	na		1905-03-26	201	na	na
cmnml47857	na	*Thyasira gouldi*	na	*Thyasira gouldi*	na	51.3133	-129.6600	British Columbia	na	Acc. 68-163	190	Aug. 1967	na	na	N. Bourne
A-5b-G3	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	63.7255	-68.5205	Frobisher Bay	M. V. Nulialjuk	na	10.5	2016-10-10	280	na	Erin Herder
A-5b-G3	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	63.7255	-68.5205	Frobisher Bay	M. V. Nulialjuk	na	10.5	2016-10-10	na	2.053	Erin Herder
A-5b-G3	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	63.7255	-68.5205	Frobisher Bay	M. V. Nulialjuk	na	10.5	2016-10-10	na	2.986	Erin Herder
A-5b-G3	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	63.7255	-68.5205	Frobisher Bay	M. V. Nulialjuk	na	10.5	2016-10-10	na	3.354	Erin Herder
A-25-G2	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	63.7224	-68.5163	Frobisher Bay	M. V. Nulialjuk	na	27.6	2016-10-11	306	na	Erin Herder
A-25-G2	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	63.7224	-68.5163	Frobisher Bay	M. V. Nulialjuk	na	27.6	2016-10-11	280	na	Erin Herder
A-28-G1	na	*Axinopsida orbiculata*	na	*Axinopsida orbiculata*	na	63.7100	-68.5211	Frobisher Bay	M. V. Nulialjuk	na	11.5	2016-10-10	na	3.069	Erin Herder
SB 1.1 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		215	3.667	Suzanne Dufour
SB 1.3 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		165	3.189	Suzanne Dufour
SB3.2 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		170	na	Suzanne Dufour
SB 3.3 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		195	3.557	Suzanne Dufour
SB 4.2 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		155	3.283	Suzanne Dufour
DB 11.3 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		na	2.364	Suzanne Dufour
NB 16.2 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		na	2.644	Suzanne Dufour
DB 9 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		205	2.630	Suzanne Dufour
NB 13 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		180	3.298	Suzanne Dufour
NB 17 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		205	3.356	Suzanne Dufour
NB 17 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		175	3.364	Suzanne Dufour
NB 19 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		na	3.093	Suzanne Dufour
NB 20 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		190	2.977	Suzanne Dufour
NB 21 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		175	3.345	Suzanne Dufour
NB 21 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		175	3.441	Suzanne Dufour
NB 22 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		205	3.032	Suzanne Dufour
NB 27 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		170	2.686	Suzanne Dufour
NB 27 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		170	2.658	Suzanne Dufour
SB 15 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		165	3.027	Suzanne Dufour
SB 1 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		na	5.041	Suzanne Dufour
SB 1 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		na	5.043	Suzanne Dufour
SB 4 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		160	3.973	Suzanne Dufour
SB 8 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		200	3.329	Suzanne Dufour

DB 2 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		180	3.618	Suzanne Dufour
DB 2 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		na	3.520	Suzanne Dufour
DB 5 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		170	3.557	Suzanne Dufour
DB 5 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		155	3.579	Suzanne Dufour
NB 1 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		200	3.036	Suzanne Dufour
SB 3.2 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		na	3.663	Suzanne Dufour
SB 3.2 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		185	3.653	Suzanne Dufour
SB 13.2 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		190	3.882	Suzanne Dufour
SB 14.2 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		205	3.776	Suzanne Dufour
SB 14.2 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		205	3.778	Suzanne Dufour
SB 15.1 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		180	4.209	Suzanne Dufour
SB 15.1 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		180	4.197	Suzanne Dufour
SB 15.3 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		190	3.866	Suzanne Dufour
DB 5.1 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		na	4.459	Suzanne Dufour
DB 5.2 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		185	4.106	Suzanne Dufour
NB 12 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		185	2.747	Suzanne Dufour
NB 12 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		180	2.777	Suzanne Dufour
NB 2 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		170	3.195	Suzanne Dufour
DB 5.3 left	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		210	4.604	Suzanne Dufour
DB 5.3 right	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	49.5000	-57.9100	Bonne Bay	na	na	30		210	4.611	Suzanne Dufour
SB 2.1 left	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		175	2.698	Suzanne Dufour
SB 2.2 right	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		165	2.912	Suzanne Dufour
DB 7.3 right	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		130	2.328	Suzanne Dufour
DB 10.2 right	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		170	2.648	Suzanne Dufour
DB 11.2 right	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		na	2.443	Suzanne Dufour
DB 12.2 right	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		195	2.630	Suzanne Dufour
DB 7 left	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		175	3.363	Suzanne Dufour
DB 10 left	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		na	3.013	Suzanne Dufour
DB 14 left	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		130	2.435	Suzanne Dufour
DB 15 left	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		180	na	Suzanne Dufour
DB 15 right	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		170	2.871	Suzanne Dufour
DB 16 left	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		165	2.787	Suzanne Dufour
DB 25 left	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		150	2.553	Suzanne Dufour
DB 25 right	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		145	2.593	Suzanne Dufour
SB 3.3 left	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		na	2.880	Suzanne Dufour
DB 5.8 left	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		155	2.898	Suzanne Dufour
DB 5.8 right	na	*Thyasira plana*	na	*Thyasira plana*	3	49.5000	-57.9167	Bonne Bay	na	na	30		180	2.913	Suzanne Dufour
Bell 6.9	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	63.6415	-68.6153	Frobisher Bay	CCGS Amundsen	na	117	2016-07-16	208	3.276	Rachelle Dove
Sag1	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	na	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	210	4.320	Rachelle Dove
Sag3	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	na	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	231	4.800	Rachelle Dove
Sag4	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	na	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	214	4.700	Rachelle Dove
Sag5	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	na	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	202	3.650	Rachelle Dove
Sag17	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	na	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	233	na	Rachelle Dove
Sag18	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	na	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	228	na	Rachelle Dove
Sag25	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	na	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	238	na	Rachelle Dove
Sag10	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	202	na	Rachelle Dove
Sag12	CMNML 2023-053	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	208	4.190	Rachelle Dove
Sag13	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	210	4.230	Rachelle Dove
Sag14	CMNML 2023-053	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	210	4.530	Rachelle Dove
Sag16	CMNML 2023-053	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	1	60.3140	-62.1960	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	208	4.930	Rachelle Dove
FB2-2.c1	na	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	2	63.6750	-68.4305	Frobisher Bay	CCGS Amundsen	na	62	2016-07-16	207	na	Rachelle Dove
Bell 1.1	na	*Thyasira plana*	na	*Thyasira plana*	3	63.5460	-68.4750	Frobisher Bay	CCGS Amundsen	na	208	2016-07-15	214	3.940	Rachelle Dove
Bell 2.1	na	*Thyasira plana*	na	*Thyasira plana*	3	63.5640	-68.5020	Frobisher Bay	CCGS Amundsen	na	204	2016-07-15	211	3.730	Rachelle Dove
Bell 3.1	na	*Thyasira plana*	na	*Thyasira plana*	3	63.5820	-68.5210	Frobisher Bay	CCGS Amundsen	na	187	2016-07-15	204	3.730	Rachelle Dove
Bell 6.7	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6420	-68.6150	Frobisher Bay	CCGS Amundsen	na	117	2016-07-16	200	3.567	Rachelle Dove
FB1-1.5	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6410	-68.6210	Frobisher Bay	CCGS Amundsen	na	135	2016-07-16	196	2.565	Rachelle Dove
FB2-2c2	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6750	-68.4300	Frobisher Bay	CCGS Amundsen	na	62	2016-07-16	175	3.735	Rachelle Dove
FB2-2c3	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6750	-68.4300	Frobisher Bay	CCGS Amundsen	na	62	2016-07-16	180	3.070	Rachelle Dove
Bell 5.6	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6427	-68.6193	Frobisher Bay	CCGS Amundsen	na	104	2016-07-16	208	na	Rachelle Dove
Bell 6.6	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6420	-68.6150	Frobisher Bay	CCGS Amundsen	na	117	2016-07-16	213	na	Rachelle Dove
FB1-1.4	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6410	-68.6210	Frobisher Bay	CCGS Amundsen	na	135	2016-07-16	197	na	Rachelle Dove
FB2-1.a1	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6640	-68.4220	Frobisher Bay	CCGS Amundsen	na	80	2016-07-16	176	na	Rachelle Dove
FB2-1.a2	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6640	-68.4220	Frobisher Bay	CCGS Amundsen	na	80	2016-07-16	204	na	Rachelle Dove
FB2-1.a3	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6640	-68.4220	Frobisher Bay	CCGS Amundsen	na	80	2016-07-16	209	na	Rachelle Dove
FB2-1.a4	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6640	-68.4220	Frobisher Bay	CCGS Amundsen	na	80	2016-07-16	208	na	Rachelle Dove
FB2-1.a5	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6640	-68.4220	Frobisher Bay	CCGS Amundsen	na	80	2016-07-16	214	na	Rachelle Dove
FB2-1.b2	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6640	-68.4220	Frobisher Bay	CCGS Amundsen	na	81	2016-07-16	195	na	Rachelle Dove
FB2-1.b3	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6640	-68.4220	Frobisher Bay	CCGS Amundsen	na	82	2016-07-16	206	na	Rachelle Dove

FB2-1.b4	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6640	-68.4220	Frobisher Bay	CCGS Amundsen	na	83	2016-07-16	190	na	Rachelle Dove
FB2-2.a1	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6750	-68.4300	Frobisher Bay	CCGS Amundsen	na	62	2016-07-16	219	na	Rachelle Dove
FB2-2.b1	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6750	-68.4300	Frobisher Bay	CCGS Amundsen	na	62	2016-07-16	208	na	Rachelle Dove
FB2-2.b2	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6750	-68.4300	Frobisher Bay	CCGS Amundsen	na	62	2016-07-16	219	na	Rachelle Dove
FB2-2.c5	na	*Thyasira plana*	na	*Thyasira plana*	3	63.6750	-68.4300	Frobisher Bay	CCGS Amundsen	na	62	2016-07-16	206	na	Rachelle Dove
FB2-2.c6	CMNML 2023-053	*Thyasira plana*	na	*Thyasira plana*	na	63.6750	-68.4300	Frobisher Bay	CCGS Amundsen	na	62	2016-07-16	206	na	Rachelle Dove
FB8.1	na	*Thyasira plana*	na	*Thyasira plana*	3	62.9230	-67.0900	Frobisher Bay	CCGS Amundsen	na	610	2016-07-17	213	na	Rachelle Dove
FB6-1.1	na	*Thyasira plana*	na	*Thyasira plana*	3	63.1100	-67.5190	Frobisher Bay	CCGS Amundsen	na	512	2016-07-15	217	na	Rachelle Dove
FB6-1.2	na	*Thyasira plana*	na	*Thyasira plana*	3	63.1100	-67.5190	Frobisher Bay	CCGS Amundsen	na	512	2016-07-15	215	na	Rachelle Dove
FB6-1.3	na	*Thyasira plana*	na	*Thyasira plana*	3	63.1100	-67.5190	Frobisher Bay	CCGS Amundsen	na	512	2016-07-15	195	na	Rachelle Dove
FB7.13	na	*Thyasira plana*	na	*Thyasira plana*	3	62.9780	-67.2820	Frobisher Bay	CCGS Amundsen	na	572	2016-07-17	212	2.361	Rachelle Dove
FB7.21	na	*Thyasira plana*	na	*Thyasira plana*	3	62.9780	-67.2820	Frobisher Bay	CCGS Amundsen	na	572	2016-07-17	186	na	Rachelle Dove
FB7.22	na	*Thyasira plana*	na	*Thyasira plana*	3	62.9780	-67.2820	Frobisher Bay	CCGS Amundsen	na	572	2016-07-17	193	na	Rachelle Dove
FB7.23	na	*Thyasira plana*	na	*Thyasira plana*	3	62.9780	-67.2820	Frobisher Bay	CCGS Amundsen	na	572	2016-07-17	196	na	Rachelle Dove
Sag11	na	*Thyasira plana*	na	*Thyasira plana*	na	60.3144	-62.1960	Saglek Basin	CCGS Amundsen	na	275	2016-07-20	195	na	Rachelle Dove
Sag15	CMNML 2023-053	*Thyasira plana*	na	*Thyasira plana*	na	60.3104	-62.1978	Saglek Basin	CCGS Amundsen	na	272	2016-07-20	192	3.100	Rachelle Dove
FB7.1	CMNML 2023-053	*Thyasira plana*	na	*Thyasira plana*	na	62.9780	-67.2820	Frobisher Bay	CCGS Amundsen	na	572	2016-07-20	na	na	Rachelle Dove
FB7.2	CMNML 2023-053	*Thyasira plana*	na	*Thyasira plana*	na	62.9780	-67.2820	Frobisher Bay	CCGS Amundsen	na	572	2016-07-20	na	na	Rachelle Dove
Bonne Bay	CMNML 2023-053	*Thyasira plana*	na	*Thyasira plana*	na	49.5000	-57.9167	Bonne Bay	na	na	30	2017-July	na	na	Suzanne Dufour
Bonne Bay	NFM MO-2889	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	na	49.5000	-57.9167	Bonne Bay	na	na	30	2016-Oct	na	na	Suzanne Dufour
Bonne Bay	NFM MO-2890	*Thyasira plana*	na	*Thyasira plana*	na	49.5000	-57.9167	Bonne Bay	na	na	30	2017-June	na	na	Suzanne Dufour
Bonne Bay	NFM MO-2891	*Thyasira plana*	na	*Thyasira plana*	na	49.5000	-57.9167	Bonne Bay	na	na	30	2017-June	na	na	Suzanne Dufour
Bonne Bay	CMNML 2023-052	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	na	49.5000	-57.9167	Bonne Bay	na	na	30	2016-Oct	na	na	Suzanne Dufour
Bonne Bay	CMNML 2023-052	*Thyasira* cf. *gouldi*	na	*Thyasira* cf. *gouldi*	na	49.5000	-57.9167	Bonne Bay	na	na	30	2016-Oct	na	na	Suzanne Dufour

Appendix B. Shell Shape Analysis

Introduction

In a previous analysis of shell shape using Elliptic Fourier Analysis, symbiotic and asymbiotic *Thyasira* cf. *gouldi* specimens from Bonne Bay (Newfoundland) could only partly be differentiated based on shell outline: while larger specimens of symbiotic *T*. cf. *gouldi* had a distinct shape, smaller specimens could not be distinguished from asymbiotic *T*. cf. *gouldi* (herein: *T. plana*) Batstone *et al.* (2014). Here, we used the same approach to compare a larger number of thyasirids from a greater number of Canadian locations, to examine the usefulness of shell outline as a distinguishing characteristic in this family. We hypothesized that shell shape would differ clearly between genera (*Axinopsida* and *Thyasira*), while species within the genus *Thyasira* could not be differentiated by their shell shape.

Materials and Methods

Thyasirid faunal collections from the Canadian Museum of Nature, arctic and sub-arctic regions (appendix A) were searched for 6 taxa found in Canadian waters: *Axinopsida orbiculata, Thyasira* cf. *dunbari, T.* cf. *equalis, T. plana, T. cf. gouldi,* and *T. gouldi s.s*. We included the type specimen of *T. dunbari* and a specimen of *T. gouldi* from the type location; other specimens labeled here as *T. gouldi* were collected off British Columbia (B.C.). All specimens considered here were assigned to taxa on the basis of anatomical features as described in Chapter 3. The taxonomic assignments indicated here are not necessarily the original; I have re-considered some of these assignments as described in Chapter 3.

The left valve of 68 individuals (Table S1) was imaged using an Olympus SZ 61 dissecting microscope and an Olympus SZX12. As in Batstone *et al*. (2014), differences in shell outline among specimens were assessed by performing statistical analyses of Elliptic Fourier

coefficients, which are landmark and size independent descriptors of shapes that are well suited for comparisons of bivalve shells (Crampton 1995). A cluster analysis in Primer 6.0 (Clarke & Gorley 2006) was run based on Euclidian distance of the first 10 harmonics to group individuals based on general differences in shape as well as to determine whether statistically significant groups could be formed.

Results and Discussion

The cluster analysis of individual shells according to their Elliptical Fourier descriptors revealed the presence of three significant groups, represented by black lines on the dendrogram in Figure S1: the first group consisted of the type specimen of *Thyasira dunbari*, the second consisted on a single specimen of *T*. cf. *dunbari* from the Gulf of St. Lawrence, while all other specimens formed the third cluster. There were no groupings by taxon, either at the genus of species level. This analysis confirms that thyasirid species cannot be determined based solely on the shell outline, and that there is significant overlap in outline across species and genera.

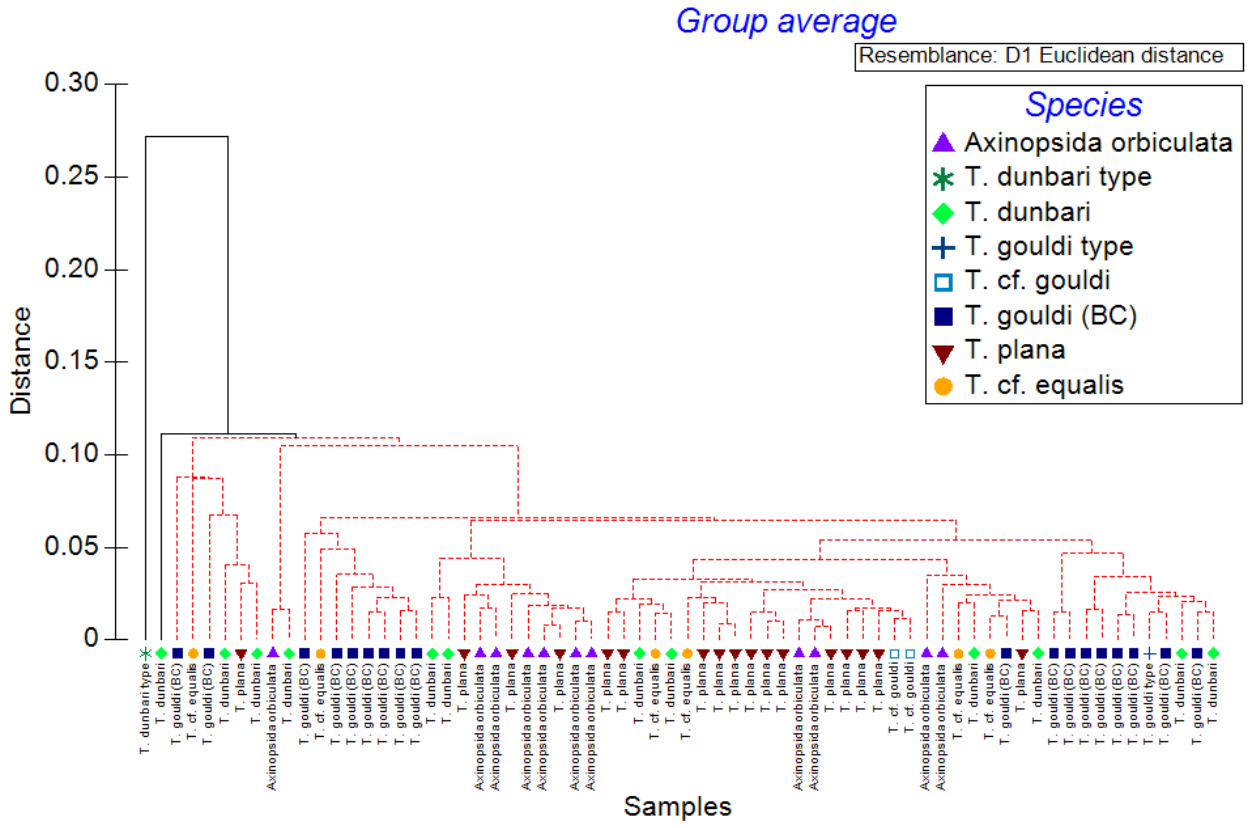

Figure S1. Dendrogram representing thyasirid specimens grouped according to their shell outline, based on Euclidean distances following Elliptic Fourier Analysis (as in Batstone *et al.* 2014). Three significant groups are represented by black lines. "T. dunbari type" refers to the type specimen, "T. dunbari" refer to *T.* cf. *dunbari*, and "*T. gouldi* type" refers to a specimen from the type location (Gulf of Maine).

Table S1. Specimen details on individuals used in the dendrogram shown in Figure S1. The number of individuals analysed from each museum lot can be found in the 4th column. When collected, shell measurements can be found in Appendix A.
*No museum lot number provided.

CMN lot number	Original Identification	Revised Identification	Individuals analysed	Location	Year
*Axinopsida_Ellesmere	*Axinopsida orbiculata*	-	5	Ellesmere Island	1983
cmnml42463	*Thyasira dunbari*	*Axinopsida orbiculata*	1	Frobisher Bay	-
cmnml79395	*Thyasira dunbari* type	-	1	Ellesmere Island	1954
cmnml66587	*Thyasira equalis*	*Thyasira* cf. *equalis*	5	Baffin Bay	1970
cmnml35052	*Thyasira gouldi*	*Thyasira plana*	3	Hudson Bay	1958
cmnml35079	*Thyasira gouldi*	*Thyasira plana*	2	Hudson Bay	1958
cmnml35177	*Thyasira gouldi*	*Thyasira plana*	1	Hudson Bay	1958
cmnml35202	*Thyasira gouldi*	*Thyasira plana*	3	Baffin Island	1958
cmnml36383	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	1	Rowley Island	1957
cmnml36467	*Thyasira gouldi*	*Thyasira plana*	2	James Bay	1959
cmnml41335	*Thyasira gouldi*	*Thyasira plana*	2	Nastapoka Island	1905
cmnml41768	*Thyasira gouldi*	*Thyasira plana*	1	Foxe Basin	1905
cmnml41792	*Thyasira gouldi*	*Thyasira plana*	1	Ungawa Bay	1947
cmnml42098	*Thyasira gouldi* type	-	1	Georges Bank	1912
cmnml45295	*Thyasira gouldi*	*Thyasira* cf. *equalis*	1	Nastapoka Sound	1963
cmnml45343	*Thyasira gouldi*	*Thyasira* cf. *dunbari*	1	Richmond Gulf	1963
cmnml47857	*Thyasira gouldi*	-	2	British Columbia	1967
cmnml70596	*Thyasira gouldi*	*Thyasira plana*	1	Creswell Bay	1962
cmnml01138	*Thyasira gouldi*	-	2	Port Simpson	1912
mnml01302	*Thyasira gouldi*	-	14	Vancouver Island	1909
cmnml36479	*Thyasira gouldi*	*Thyasira* cf. *gouldi*	1	Hudson Bay	1959
cmnml36479	*Thyasira gouldi*	*Thyasira plana*	1	Hudson Bay	1959
cmnml73960	*Thyasira gouldi*	*Thyasira* cf. *dunbari*	4	St. Lawrence R.	1971
cmnml43439	*Axinopsida orbiculata*	-	5	Greenland	1905
*arctique2010	*Thyasira* sp.	*Thyasira* cf. *dunbari*	2	Lancastor Sound	2010
*arctique2011	*Thyasira* sp.	*Thyasira* cf. *dunbari*	5	Dease Strait	2011

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