



NE Atlantic Marine Biological Analytical Quality Control Scheme

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Ring Test Bulletin – RTB#66: deuterostomes (Echinodermata, Cephalochordata, Ascidiacea)



Tim Worsfold, Ceri Miller, Nicola Pennisi David Hall Søren Pears (Images)



APEM Ltd. July 2024 *E-mail: <u>nmbaqc@apemItd.co.uk</u>*

RING TEST DETAILS

Ring Test #66 (Year 30) Type/Contents – Targeted, deuterostomes (Echinodermata, Cephalochordata, Ascidiacea) Circulated – 08/04/24 Results deadline – 31/05/24 Number of Subscribing Laboratories – 21 Number of Participating Laboratories – 19 Number of Results Received – 17* *multiple data entries per laboratory permitted

Summary of differences

				Total differences			
Specimen	Genus	Species	Condition / Size	for 17	returns		
				Genus	Species		
RT6601	Ophiocten	gracilis	fair, medium	3	12		
RT6602	Astropecten	irregularis	good, small	5	5		
RT6603	Amphilepis	norvegica	good, medium	6	6		
RT6604	Ophiactis	balli	good, small	1	1		
RT6605	Psammechinus	miliaris	good, medium	1	1		
RT6606	Echinocyamus	pusillus	good, small	0	0		
RT6607	Acrocnida	brachiata	good, medium	1	1		
RT6608	Ophiura	albida	good, medium	0	0		
RT6609	Amphiura	filiformis	fair, medium	0	0		
RT6610	Amphipholis	squamata	good, medium	1	1		
RT6611	Amphiura	chiajei	good, medium	0	0		
RT6612	Ocnus	planci	good, medium	2	6		
RT6613	Leptosynapta	bergensis	fair, medium	0	4		
RT6614	Echinocardium	cordatum	good, medium	0	0		
RT6615	Labidoplax	buskii	fair, medium	0	0		
RT6616	Branchiostoma	lanceolatum	good, medium	0	0		
RT6617	Asterocarpa	humilis	good, medium	10	10		
RT6618	Molgula	complanata	good, medium	3	7		
RT6619	Didemnum	vexillum	fair, small portions	0	1		
RT6620	Styela	canopus	fair, medium	7	13		
RT6621	Polycarpa	pomaria	good, medium	6	9		
RT6622	Botrylloides	violaceus	fair, small portions	4	7		
RT6623	Ascidiella	scabra	good, medium	5	11		
RT6624	Dendrodoa	grossularia	good, medium	4	4		
RT6625	Microcosmus	claudicans	good, medium	11	11		
			Total differences	70	110		
			Average differences /lab.	4.1	6.5		

Figure A. The number of differences from the AQC identification of specimens distributed in RT66 for each of the participating laboratories. Arranged in order of increasing number of differences (by specific followed by generic errors).



	RT6601	RT6602	RT6603	RT6604	RT6605	RT6606	RT6607	RT6608	RT6609	RT6610	RT6611	RT6612
	Ophiocten	Astropecten	Amphilepis		Psammechinus	Echinocyamus	Acrocnida		Amphiura	Amphipholis	Amphiura	
Taxon	gracilis	irregularis	norvegica	Ophiactis balli	miliaris	pusillus	brachiata	Ophiura albida	filiformis	squamata	chiajei	Ocnus planci
BI_3002	Ophiura robusta					- [pusillis]						- brunneus
BI_3003	- sericeum											
BI_3004	- affinis											
BI_3005												
BI_3006	- affinis											
BI_3007	- affinis		Amphiura filiformis									
BI_3008		Luidia sarsii										
BI_3009												
BI_3010	- affinis		Ophiocomina nigra									- brunneus
BI_3011	- affinis	Asterias rubens										- brunneus
BI_3012		Asterias rubens										
BI_3013	- affinis											
BI_3015	- affinis		Amphiura securigera									- brunneus
BI_3016	Ophiura sarsi		Amphiura filiformis									
BI_3018	Ophiura robusta	Goniasteridae juv.	Ophiactis balli	Ophiothrix fragilis	Gracilechinus elegans		Amphiura incana			Amphiuridae juv.		Cucumaria frondosa
BI_3019	- affinis	Asterias rubens	Amphiura filiformis									
BI_3020												Aslia lefevrei

	RT6613	RT6614	RT6615	RT6616	RT6617	RT6618	RT6619	RT6620	RT6621	RT6622	RT6623	RT6624	RT6625
	Leptosynapt	Echinocardium	Labidoplax	Branchiostoma	Asterocarpa	Molgula	Didemnum	Styela	Polycarpa	Botrylloides	Ascidiella	Dendrodoa	Microcosmus
Taxon	a bergensis	cordatum	buskii	lanceolatum	humilis	complanata	vexillum	canopus	pomaria	violaceus	scabra	grossularia	claudicans
BI_3002			- [buski]		Phallusia mammillata	- manhattensis		Pyura microcosmos	Styela coriacea	Diplosoma listerianum			Styela clava
BI_3003							- helgolandicum						
BI_3004					Styela coriacea	- citrina		- clava	Styela partita	- leachii	- aspersa		Pyura microcosmus
BI_3005													Styela clava
BI_3006								- clava			- aspersa		
BI_3007	- inhaerens	- [chordatum]	- [buski]		Polycarpa pomaria	[Mogula] [complanta]		- clava					Styela clava
BI_3008					Styela canopus				- fibrosa		- aspersa		Styela clava
BI_3009								Pyura tessellata			Ascidia obliqua		
BI_3010					Ascidiella aspersa	- citrina		- clava	- fibrosa	Aplidium nordmanni		Styela canopus	Styela clava
BI_3011	- inhaerens				Pyura microcosmus	Ascidia obliqua		Pyura microcosmus					
BI_3012					Polycarpa pomaria			Phallusia mammillata	Pyura microcosmus	Aplidium urgorrii	- aspersa		Styela clava
BI_3013					Polycarpa pomaria			- clava	Asterocarpa humilis		Ascidia mentula		
BI_3015	- inhaerens				Polycarpa pomaria	Dendrodoa grossularia		- coriacea	Styela clava	- leachii	Ascidia mentula	Phallusia mammillata	Styela clava
BI_3016	- inhaerens		- [buski]		Corella eumyota	Ascidiella scabra		Polycarpa pomaria	Styela clava		Corella parallelogramma		Styela clava
BI_3018						- citrina		Polycarpa pomaria	- fibrosa	- leachii	Ascidia obliqua	Stolonica socialis	Pyura microcosmus
BI_3019					Molgula manhattensis/ socialis			Polycarpa pomaria	Dendrodoa grossularia	Disstaplia rosea	- aspersa	Ascidiella scabra	Styela clava
BI_3020											- aspersa		

	Taxon	BI_3002	BI_3003	BI_3004	BI_3005	BI_3006	BI_3007	BI_3008	BI_3009	BI_3010	BI_3011
RT6601	Ophiocten gracilis	Ophiura robusta	- sericeum	- affinis		- affinis	- affinis			- affinis	- affinis
RT6602	Astropecten irregularis							Luidia sarsii			Asterias rubens
RT6603	Amphilepis norvegica						Amphiura filiformis			Ophiocomina nigra	
RT6604	Ophiactis balli										
RT6605	Psammechinus miliaris										
RT6606	Echinocyamus pusillus	- [pusillis]									
RT6607	Acrocnida brachiata										
RT6608	Ophiura albida										
RT6609	Amphiura filiformis										
RT6610	Amphipholis squamata										
RT6611	Amphiura chiajei										
RT6612	Ocnus planci	- brunneus								- brunneus	- brunneus
RT6613	Leptosynapta bergensis						- inhaerens				- inhaerens
RT6614	Echinocardium cordatum						- [chordatum]				
RT6615	Labidoplax buskii	- [buski]					- [buski]				
RT6616	Branchiostoma Ianceolatum										
RT6617	Asterocarpa humilis	Phallusia mammillata		Styela coriacea			Polycarpa pomaria	Styela canopus		Ascidiella aspersa	Pyura microcosmus
RT6618	Molgula complanata	- manhattensis		- citrina			[Mogula] [complanta]			- citrina	Ascidia obliqua
RT6619	Didemnum vexillum		- helgolandicum								
RT6620	Styela canopus	Pyura microcosmos		- clava		- clava	- clava		Pyura tessellata	- clava	Pyura microcosmus
RT6621	Polycarpa pomaria	Styela coriacea		Styela partita				- fibrosa		- fibrosa	
RT6622	Botrylloides violaceus	Diplosoma listerianum		- leachii						Aplidium nordmanni	
RT6623	Ascidiella scabra			- aspersa		- aspersa		- aspersa	Ascidia obliqua		
RT6624	Dendrodoa grossularia									Styela canopus	
RT6625	Microcosmus claudicans	Styela clava		Pyura microcosmus	Styela clava		Styela clava	Styela clava		Styela clava	

	Taxon	BI_3012	BI_3013	BI_3015	BI_3016	BI_3018	BI_3019	BI_3020
RT6601	Ophiocten gracilis		- affinis	- affinis	Ophiura sarsi	Ophiura robusta	- affinis	
RT6602	Astropecten irregularis	Asterias rubens				Goniasteridae juv.	Asterias rubens	
RT6603	Amphilepis norvegica			Amphiura securigera	Amphiura filiformis	Ophiactis balli	Amphiura filiformis	
RT6604	Ophiactis balli					Ophiothrix fragilis		
RT6605	Psammechinus miliaris					Gracilechinus elegans		
RT6606	Echinocyamus pusillus							
RT6607	Acrocnida brachiata					Amphiura incana		
RT6608	Ophiura albida							
RT6609	Amphiura filiformis							
RT6610	Amphipholis squamata					Amphiuridae juv.		
RT6611	Amphiura chiajei							
RT6612	Ocnus planci			- brunneus		Cucumaria frondosa		Aslia lefevrei
RT6613	Leptosynapta bergensis			- inhaerens	- inhaerens			
RT6614	Echinocardium cordatum							
RT6615	Labidoplax buskii				- [buski]			
RT6616	Branchiostoma lanceolatum							
RT6617	Asterocarpa humilis	Polycarpa pomaria		Polycarpa pomaria	Corella eumyota		Molgula manhattensis/socialis	
RT6618	Molgula complanata			Dendrodoa grossularia	Ascidiella scabra	- citrina		
RT6619	Didemnum vexillum							
RT6620	Styela canopus	Phallusia mammillata	- clava	- coriacea	Polycarpa pomaria	Polycarpa pomaria	Polycarpa pomaria	
RT6621	Polycarpa pomaria	Pyura microcosmus		Styela clava	Styela clava	- fibrosa	Dendrodoa grossularia	
RT6622	Botrylloides violaceus	Aplidium urgorrii		- leachii		- leachii	Disstaplia rosea	
RT6623	Ascidiella scabra	- aspersa	Ascidia mentula	Ascidia mentula	Corella parallelogramma	Ascidia obliqua	- aspersa	- aspersa
RT6624	Dendrodoa grossularia			Phallusia mammillata		Stolonica socialis	Ascidiella scabra	
RT6625	Microcosmus claudicans	Styela clava		Styela clava	Styela clava	Pyura microcosmus	Styela clava	

Introduction to Ring Test 66

RT66 was targeted on deuterostomes (Echinodermata, Cephalochordata, Ascidiacea).

The aims of the Ring Test (RT) exercises are to examine consistency of species identifications, to highlight identification problems and literature updates and to familiarise participants with species that they may not have previously encountered (Worsfold & Hall, 2017). The results are not used to assess the performance of a laboratory and the graph with categories for numbers of identification differences is provided for interest only. Species are selected to improve our understanding of the fauna. This may be through inclusion of species not previously sent: RT66 included fourteen species never previously sent. Species not yet photographed according to current protocols are also selected. Recently, species have also been selected to provide insights to help with the development of a taxonomic discrimination protocol, as detailed under family headings in the discussion section below. The geographical scope was originally British waters. It is now expanded to include northern Europe and specimens may be included from further afield if the species is known from northern Europe or likely to be found there in future.

LabCodes are abbreviated in this report to exclude the Scheme year, *e.g.* BI_3001 = Lab 01. An additional terminal character has been added within each LabCode (small case sequential letters) to permit multiple data entries from each laboratory, *i.e.* two participants from laboratory 01 would be coded as Lab 01a & Lab 01b. For details of your LabCode please contact your Scheme representative or APEM Ltd.

Worsfold, T.M. & Hall, D.J., 2017. *Benthic Invertebrate component - Ring Test Protocol*. Report to the NMBAQC Scheme participants. 6pp, August 2017.

Specimen Images and Detailed Breakdown of Identifications

Basic differences are given below. More detail may be available in the later 'taxonomic and Identification policy considerations' section.

(Figure codes: A=anterior; P=posterior; L=lateral; D=dorsal; V=ventral). The codes in brackets following the species names below the figures are sample identification codes to allow tracking of sources of specimens.

RT6601 – Ophiocten gracilis (G.O. Sars, 1872) (Figures 1a, 1b)

Substratum: Mud. Salinity: Full (Euhaline). Depth: Circalittoral (Lower Shelf). Geography: northern Scotland. Condition: Fair. Size: Medium. Specimens from nine samples.



Fig. 1a. Ophiocten gracilis (RT6601, 62447) – D

Three generic and twelve specific differences: Labs 04, 06, 07, 10, 11, 13, 15 and 19 identified as *Ophiocten affinis* (Figures 1c, 1d) (which has oral shields twice as long as broad and a more rounded edge to the disk, as seen from the side); Lab 03 identified as *Ophiocten sericeum* (Figures 1c, 1d) (which has oral shields slightly longer than broad); Lab 16 identified as *Ophiura sarsi*, currently *O. sarsii* (Figure 1e, 1f); Labs 02 and 18 identified as *Ophiura robusta* (no material available) (both of which have arm combs separated above the arms).



Fig. 1b. Ophiocten gracilis (RT6601, 62447) – V



Fig. 1d. Ophiocten affinis (P2081, 60034) – V



Fig. 1f. Ophiura sarsi (P1903, 59911) – V



Fig. 1c. Ophiocten affinis (P2081, 60034) – D



Fig. 1e. Ophiura sarsi (P1903, 59911) – D

RT6602 – Astropecten irregularis (Pennant, 1777) (Figures 2a, 2b, 2d, 2g, 2j)

Substratum: Sand. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: North Sea. Condition: Good. Size: Small. Specimens from twelve samples.



Fig. 2a. Astropecten irregularis (RT6602, 74857) –

D

Fig. 2b. Astropecten irregularis (RT6602, 74857) –

Five generic and specific differences: Labs 11, 12 and 19 identified as *Asterias rubens* (Figures 2c, 2e, 2h,) (which has relatively longer arms and fewer, more evenly-sized spines at this size); Lab 08 identified as *Luidia sarsii* (Figures 2f, 2i, 2k) (which has paxillae with more pointed spines); Lab 18 identified as Goniasteridae juv. (no material available) (which have sucking disks on the tube feet).

It is recommended that laboratories attempt species level identification of all specimens.



Fig. 2c. Asterias rubens (14612, 76706) – D



Fig. 2d. Astropecten irregularis (P1223_58270) – D



Fig. 2e. Asterias rubens (414083, 54977) – D



Fig. 2f. Luidia sarsii (P1223_58270) – D



Fig. 2h. Asterias rubens (414083, 54977) – D



Fig. 2j. Astropecten irregularis (P1223_58270) – paxillae



Fig. 2g. Astropecten irregularis (413885, 55007) –



Fig. 2i. *Luidia sarsi* (P12697, 75460) – D



Fig. 2k. Luidia sarsii (P1223_58270) – paxillae

RT6603 – Amphilepis norvegica (Ljungman, 1865) (Figures 3a, 3b)

Substratum: Mud. Salinity: Full (Euhaline). Depth: Circalittoral (Lower Shelf). Geography: Norway. Condition: Good. Size: Medium. Specimens from four samples.



Six generic and specific differences: Labs 07, 10 and 19 identified as Amphiura filiformis (Figures 9a, 9b); Lab 15 identified as Amphiura securigera (Figures 3c, 3d) (both of which have paired infradental papillae at the apex of the jaw); Lab 18 identified as Ophiactis balli (Figures 4a, 4b) (which has spines on the disk and a heart-shaped single infradental papilla); Lab 10 identified as Ophiocomina nigra (Figures 3e, 3f) (which has the disk covered in small granules, lacks infradental papillae and has tooth papillae).

Fig. 3a. Amphilepis norvegica_(RT6603, 59427) - D







Fig. 3d. Amphiura securigera (413532, 10109) – V

Fig. 3b. Amphilepis norvegica (RT6603, 59427) – V Fig. 3c. Amphiura securigera (413532, 10109) – D



Fig. 3e. Ophiocomina nigra (P4206, 64162) – D



Fig. 3f. Ophiocomina nigra (P4206, 64162) – V

RT6604 – Ophiactis balli (W. Thompson, 1840) (Figures 4a-b)

Substratum: Diamicton. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: eastern Scotland. Condition: Good. Size: Small. Specimens from two samples.



identified as *Ophiothrix fragilis* (Figures 4c, 4d) (which has spiny arm spines and lacks infradental papillae but has an apical group of small tooth papillae).

One generic and specific difference: Lab 18

Fig. 4a. Ophiactis balli (RT6604, 72697) - D



Fig. 4b. *Ophiactis balli* (RT6604, 72697) – V



Fig. 4c. Ophiothrix fragilis (413154, 40692) – D



Fig. 4d. Ophiothrix fragilis (413154, 40692) - V

RT6605 – Psammechinus miliaris (P.L.S. Müller, 1771) (Figures 5a, 5b, 5g)

Substratum: Diamicton. Salinity: Full (Euhaline). Depth: Infralittoral. Geography: southeast England. Condition: Good. Size: Medium. Specimens from nine samples.



One generic and specific difference: Lab 18 identified as *Gracilechinus elegans* (Figures 5c, 5d, 5h) (Figures 5e, 5f, 5i show *G. acutus*) (both of which have globular pedicellariae with tubular blades and only 2-3 teeth on each side of the valves).

Fig. 5a. Psammechinus miliaris (RT6605, 73700)



Fig. 5b. *Psammechinus miliaris* (RT6605, 73700) – V



Fig. 5c. *Gracilechinus elegans* (P3132, 62474) – D



Fig. 5d. Gracilechinus elegans (P3132, 62474) – Fig. 5e. Gracilechinus acutus (411344, 35125) – V



Fig. 5f. Gracilechinus acutus (411344, 35125) – Fig. 5g. Psammechinus miliaris (410875, 33622) –





100 µm

globular pedicellaria valve



globular pedicellaria valves



RT6606 – Echinocyamus pusillus (O.F. Müller, 1776) (Figures 6a, b)

Substratum: Sand. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: North Sea. Condition: Good. Size: Small. All specimens from one sample.



Fig. 6a. Echinocyamus pusillus (RT6606, 74916)



Fig. 6b. Echinocyamus pusillus (RT6606, 74916) - V

RT6607 – Acrocnida brachiata (Montagu, 1804) (Figures 7a-b)

Substratum: Sand. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: North Sea. Condition: Good. Size: Medium. Specimens from two samples.

plates).



Fig. 7a. Acrocnida brachiata (RT6607, 74928) - D

No generic or specific differences recorded.

One generic and specific difference: Lab 18 identified as *Amphiura incana* (no material available) (which lacks tubercles on the disk scales and lacks a groove on the ventral arm



Fig. 7b. Acrocnida brachiata (RT6607, 74928) – V

<u>RT6608 – Ophiura albida Forbes, 1839 (Figures 8a-b)</u>

Substratum: Sand. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: southeast England. Condition: Good. Size: Medium. Specimens from three samples.



Fig. 8a. Ophiura albida (RT6608, 70470) – D



Fig. 8b. Ophiura albida (RT6608, 70470) – V

No generic or specific differences recorded.

RT6609 – Amphiura filiformis (O.F. Müller, 1776) (Figures 9a, b)

Substratum: Mud. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: northwest England. Condition: Fair. Size: Medium. All specimens from one sample.



Fig. 9a. Amphiura filiformis (RT6609, 54956) -



Fig. 9b. Amphiura filiformis (RT6609, 54956) –

RT6610 – Amphipholis squamata (Delle Chiaje, 1828) (Figures 10a-b)

Substratum: Diamicton. Salinity: Full (Euhaline). Depth: Depth: Infralittoral. Geography: north of Ireland. Condition: Good. Size: Medium. All specimens from one sample.



Fig. 10a. Amphipholis squamata (RT6610, 8307) – D

One generic and specific difference: Lab 18 identified as Amphiuridae juv. It is recommended that laboratories attempt species level identification of all specimens.

No generic or specific differences recorded.

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Fig. 10b. Amphipholis squamata (RT6610, 8307) – V

RT6611 – Amphiura chiajei Forbes, 1843 (Figures 11a, b)

Substratum: Mud. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: western Scotland. Condition: Good. Size: Medium. All specimens from one sample.



Fig. 11a. Amphiura chiajei (RT6611, 8869) – D



Fig. 11b. Amphiura chiajei (RT6611, 8869) – V

No generic or specific differences recorded.

RT6612 – Ocnus planci (Brandt, 1835) (Figures 12a, 12c, 12d)

Substratum: Diamicton. Salinity: Full (Euhaline). Depth: Depth: Infralittoral. Geography: southeast England. Condition: Good. Size: Medium. All specimens from one sample.



Two generic and six specific differences: Labs 02, 10, 11 and 15 identified as *Ocnus brunneus* (Figures 12b, 12e, 12f) (which has tube feet in a single zig-zag row and convex outer layer spicules); Lab 20 identified as *Aslia lefevrei* (no material available) (which has cup-shaped outer layer spicules and inner spicules with only four holes); Lab 18 identified as *Cucumaria frondosa* (no material available) (which has few spicules, with small tubercles).

Fig. 12a. Ocnus planci (RT6612; 72251) - L



Fig. 12b. Ocnus brunneus (P2322, 60664) – L



Fig. 12c. Ocnus planci (RT6612; 72251) – Outer layer spicules



Fig. 12d. Ocnus planci (RT6612; 72251) – Inner layer spicules



Fig. 12e. Ocnus brunneus (P2322, 60664) – Outer layer spicules



Fig. 12f. Ocnus brunneus (P2322, 60664) – Inner layer spicules

<u> RT6613 – Leptosynapta bergensis (Östergren, 1905) (Figures 13a, 13c)</u>

Substratum: Mud. Salinity: Full (Euhaline). Depth: Infralittoral (Upper Shelf). Geography: western Scotland. Condition: Fair. Size: Medium. Specimens from eight samples.



Four specific differences: Labs 07, 11, 15 and 16 identified as *Leptosynapta inhaerens* (Figures 13b, 13d) (which has short muscle spicules and tentacle digits longest at the distal end, as well as anchor spicules that are only slightly longer than their anchor plates).

Fig. 13a. Leptosynapta bergensis (RT6613; 66258) – L



Fig. 13b. *Leptosynapta inhaerens* (P1903, 59926) – L



Fig. 13c. Leptosynapta bergensis (RT6613; 66258) – body wall spicules



Fig. 13d. *Leptosynapta inhaerens* (414075, 55069) – body wall spicules

RT6614 – Echinocardium cordatum (Pennant, 1777) (Figures 14a, b)

Substratum: Sand. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: Netherlands. Condition: Good. Size: Medium. Specimens from seven samples.



Fig. 14a. Echinocardium cordatum (RT6614, 73541) – D



Fig. 14b. Echinocardium cordatum (RT6614, 73541)

No generic or specific differences recorded.

RT6615 – Labidoplax buskii (MacIntosh, 1866) (Figures 15a, 15b, 15c)

Substratum: Mud. Salinity: Full (Euhaline). Depth: Depth: Infralittoral. Geography: western Scotland. Condition: Fair. Size: Medium. All specimens from one sample.



Fig. 15a. *Labidoplax buskii* (RT6615, 66260) – L





No generic or specific differences recorded.

Lab 07 spelled the specific name 'buski'.

Fig. 15b. *Labidoplax buskii* (RT6615, 66260) – Anchor spicule

Fig. 15c. Labidoplax buskii (RT6615, 66260) – Plate spicule

RT6616 – Branchiostoma lanceolatum (Pallas, 1774) (Figure 16a)

Substratum: Diamicton. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: North Sea. Condition: Good. Size: Medium. Specimens from three samples.



Fig. 16a. Branchiostoma lanceolatum (RT6616, 71874) – L

No generic or specific differences recorded.

RT6617 – Asterocarpa humilis (Heller, 1878) (Figures 17a, 17e)

Substratum: Hard Substrata. Salinity: Full (Euhaline). Depth: Depth: Infralittoral. Geography: northern Scotland. Condition: Good. Size: Medium. Specimens from three samples. Non-native species first recognised in UK waters in 2009.



Fig. 17a. Asterocarpa humilis (RT6617, 75326) - L

Eleven generic and specific differences: Lab 04 identified as *Styela coriacea* (no material available); Labs 04 and 08 identified as *Styela canopus* (Figures 20a, 20d); Lab 19 identified as *Molgula manhattensis/socialis* (Figures 17b, 18d, 18f) (all of which have simple gonads); Labs 07, 12 and 15 identified as *Polycarpa pomaria* (Figures 21a, 21c) (which has more scattered gonads); Lab 11 identified as *Pyura microcosmus* (Figures 17c, 17f); Lab 10 identified as *Ascidiella aspersa* (Figures 23b, 23f, 23j, 23n, 23r); Lab 02 identified as *Phallusia mammillata* (no material available) (all of which have simple ovaries); Lab 16 identified as *Corella eumyota* (Figures 17d, 17g) (which has spiral stigmata).



Fig. 17b. *Molgula manhattensis/socialis* (RT6014 ; 2233.2, 64704) – L



Fig. 17d. Corella eumyota (P529_58324) – L

Lab 13 transposed Specimens 17 and 21.



Fig. 17c. Pyura microcosmus (412692, 39329) - L



Fig. 17e. Asterocarpa humilis (P529, 58341) – Interior





Fig. 17f. *Pyura microcosmus* (412692, 39329) – Fig. 17g. *Corella eumyota* (P529_58324) – Interior Interior

<u>RT6618 – Molgula complanata Alder & Hancock, 1870 (Figures 18a, 18c, 18e)</u> Substratum: Hard Substrata. Salinity: Full (Euhaline). Depth: Depth: Infralittoral. Geography: northern Scotland. Condition: Good. Size: Medium. Specimens from four samples.



Fig. 18a. *Molgula complanata* (RT6618, 75357) – L

Three generic and seven specific differences: Lab 2 identified as *Molgula manhattensis* (Figures 17b, 18d, 18f) Labs 04, 10 and 18 identified as *Molgula citrina* (Figures 18b, 18g) (both of which have well developed branchial folds and posteriorly directed oviducts); Lab 15 identified as *Dendrodoa grossularia* (Figures 24a, 24b) (which has longitudinal stomach grooves); Lab 16 identified as *Ascidiella scabra* (Figures 23a, 23e, 23i, 23m, 23q, 24c); Lab 11 identified as *Ascidia obliqua* (no material available; Figures 23c, 23g, 23k, 23o show *A. mentula*) (both of which have rectangular stigmata).



Fig. 18b. *Molgula citrina* (P2188.3, 63444) – L (test removed)

Lab 07 spelled the specific name 'complanta'.



Fig. 18c. *Molgula complanata*(RT6618, 75357) – L (test removed)



Fig. 18d. *Molgula manhattensis/socialis* (RT6618, 75357) – L (test removed)



Fig. 18f. *Molgula manhattensis/socialis* (RT6618, 75357) – (internal)



Fig. 18e. *Molgula complanata* (RT6618, 75357) – (internal)



Fig. 18g. *Molgula citrina* (P2188.3, 63444) – (internal)

RT6619 – Didemnum vexillum Kott, 2002 (Figures 19a, 19b)

Substratum: Hard Substrata. Salinity: Full (Euhaline). Depth: Depth: Infralittoral. Geography: eastern Ireland. Condition: Fair. Size: Medium portions. Specimens from three samples. Non-native species first recognised in UK waters in 2009.



Fig. 19a. *Didemnum vexillum* (RT6619, 70730) - D

One specific difference: Lab 03 identified as *Didemnum helgolandicum*, a synonym of *D. maculosum* (no material available) (which has larvae with only two adhesive papillae – see below).



Fig. 19b. *Didemnum vexillum* (RT6619, 70730) – Larva

RT6620 - Styela canopus (Savigny, 1816) (Figures 20a, 20d)

Substratum: Hard Substrata. Salinity: Full (Euhaline). Depth: Depth: Infralittoral. Geography: northern Scotland. Condition: Fair. Size: Medium. Specimens from two samples. Cryptogenic species.



Fig. 20a. Styela canopus (RT6620, 73236) - L



Fig. 20b. Styela clava (P848_58029) – L

Seven generic and thirteen specific differences: Lab 15 identified as *Styela coriacea* (no material available) (which has only one gonad on each side); Labs 04, 06, 07, 10 and 13 identified as *Styela clava* (Figures 20b, 25b, 25d) (which has multiple gonads); Labs 16, 18 and 19 identified as *Polycarpa pomaria* (Figures 21a, 21c) (which has multiple scattered gonads); Labs 02 and 11 identified as *Pyura microcosmos* (Figures 17c, 17f); Lab 09 identified as *Pyura tessellata* (Figures 20c, 20e); Lab 12 identified as *Phallusia mammillata* (no material available) (all of which lack longitudinal stomach folds).



Fig. 20c. Pyura tessellata (413531_43499) – D



Fig. 20d. *Styela canopus* (RT6620, 73236) – internal



Fig. 20e. Pyura tessellata (413531_43499) – internal

RT6621 – Polycarpa pomaria (Savigny, 1816) (Figures 21a, 21c)

Substratum: Diamicton. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: southeast England. Condition: Good. Size: Medium. All specimens from one sample.



Fig. 21a. *Polycarpa pomaria* (RT6621, 11347) – L

Seven generic and ten specific differences: Labs 08, 10 and 18 identified as *Polycarpa fibrosa* (Figures 21b, 21d) (which has numerous hairlike processes on the surface of the test); Lab 02 identified as *Styela coriacea* (no material available); Lab 04 identified as *Styela partita*, a synonym of *Styela canopus* (Figures 20a, 20d) (which has two gonads on each side); Lab 15 and 16 identified as *Styela clava* (Figures 20b, 25b, 25d) (which has a distinct narrow stalk); Lab 19 identified as *Dendrodoa grossularia*, (Figures 24a, 24b) (which has indistinct branchial folds); Lab 12 identified as *Pyura microcosmos* (Figures 17c, 17f) (which lacks longitudinal stomach folds).



Fig. 21b. *Polycarpa fibrosa* (413557, 10432) – L

Lab 13 transposed Specimens 17 and 21.



Fig. 21c. Polycarpa pomaria (P1301, 58454) – internal



Fig. 21d. Polycarpa fibrosa (413667, 11365) – internal

RT6622 – Botrylloides violaceus Oka, 1927 (Figures 22a, 22d)

Substratum: Hard Substrata. Salinity: Full (Euhaline). Depth: Depth: Infralittoral. Geography: western Ireland. Condition: Fair. Size: Medium portions. All specimens from one sample. Non-native species first recognised in UK waters in 2004.



Fig. 22a. Botrylloides violaceus (RT6622, 70707) – D



Fig. 22b. *Botrylloides leachii* (413667, 43381) – D

Four generic and seven specific differences: Labs 04, 15 and 18 identified as *Botrylloides leachii* (Figure 22b) (which has larvae with only eight ampullae); Lab 02 identified as *Diplosoma listerianum* (Figures 22c, 22e) (which lacks longitudinal stomach folds); Lab 10 identified as *Aplidium nordmanni* (no material available, Figure 22f shows *Aplidium pallidum*) (which has oral siphons with six lobes); Lab 12 identified as *Aplidium urgorri* (no material available) (which has oral siphons with eight lobes); Lab 19 identified as *Disstaplia rosea* (*Distaplia rosea*) (no material available) (which has six-lobed oral siphons).



Fig. 22c. *Diplosoma listerianum* (P2188.1, 61888) – D



Fig. 22d. *Botrylloides violaceus* (RT6622, 70707) – Larva



Fig. 22f. Aplidium pallidum (P2188.3, 63688) – D



Fig. 22e. *Diplosoma listerianum* (P4652.1, 66379) – Larva

RT6623 – Ascidiella scabra (Müller, 1776) (Figures 23a, 23e, 23i, 23m, 23q)

Substratum: Hard Substrata. Salinity: Full (Euhaline). Depth: Depth: Infralittoral. Geography: northern Scotland. Condition: Good. Size: Medium. Specimens from two samples.



Fig. 23a. Ascidiella scabra (RT6623, 75358) - L



Fig. 23b. Ascidiella aspersa (413446, 43076) – L



Fig. 23d. Corella parallelogramma (P4652.1, 66392) – L

Five generic and eleven specific differences: Labs 04, 06, 08, 12, 19 and 20 identified as *Ascidiella aspersa* (Figures 23b, 23f, 23j, 23n, 23r) (which has oral tentacles less numerous than internal longitudinal branchial bars); Labs 13 and 15 identified as *Ascidia mentula* (Figures 23c, 23g, 23k, 23o); Labs 09 and 18 identified as *Ascidia obliqua* (no material available) (both of which have papillae on the longitudinal branchial vessels); Lab 16 identified as *Corella parallelogramma* (Figures 23d, 23h, 23l, 23p) (which has spiral stigmata).



Fig. 23c. Ascidia mentula (P2188 3 63692) – L



Fig. 23e. Ascidiella scabra (RT6623, 75358) – test removed



Fig. 23f. Ascidiella aspersa (413446, 43076) – test removed



Fig. 23h. Corella parallelogramma (P4652.1, 66392) – test removed



Fig. 23g. Ascidia mentula (P2188_3_63692) – test removed



Fig. 23i. Ascidiella scabra (RT6623, 75358) – internal



Fig. 23j. Ascidiella aspersa (413446, 43076) – internal



Fig. 23k. Ascidia mentula (P9317, 74341) – internal



Fig. 23I. Corella parallelogramma (P4652.1, 66392) – internal



Fig. 23n. Ascidiella aspersa (413446, 43076) – branchial bars



Fig. 23p. *Corella parallelogramma* (P4652.1, 66392) – stigmata



Fig. 23m. Ascidiella scabra (RT6623, 75358) – branchial bars



Fig. 230. Ascidia mentula (P9317, 74341) – branchial bars



Fig. 23q. Ascidiella scabra (RT6623, 75358) – oral tentacles



Fig. 23r. Ascidiella aspersa (413446, 43076) – oral tentacles

RT6624 – Dendrodoa grossularia (Van Beneden, 1846) (Figures 24a, 24b)

Substratum: Diamicton. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: southeast England. Condition: Good. Size: Medium. All specimens from one sample.



Fig. 24a. Dendrodoa grossularia (RT6624; 67163) –

Four generic and specific differences: Lab 18 identified as *Stolonica socialis* (no material available) (which has zooids linked by basal stolons); Lab 10 identified as *Styela canopus* (Figures 20a, 20d) (which has distinct branchial folds); Lab 19 identified as *Ascidiella scabra* (Figures 23a, 23e, 23i, 23m, 23q, 24c); Lab 15 identified as *Phallusia mammillata* (no material available) (both of which lack longitudinal stomach folds).



Fig. 24b. *Dendrodoa grossularia* (RT6624; 67163) – internal



Fig. 24c. *Ascidiella scabra* (413667, 43400) – D

RT6625 – Microcosmus claudicans (Savigny, 1816) (Figures 25a, 25c)

Substratum: Diamicton. Salinity: Full (Euhaline). Depth: Circalittoral (Upper Shelf). Geography: southeast England. Condition: Good. Size: Medium. Specimens from two samples.



Ten generic and specific differences: Labs 04 and 18 identified as *Pyura microcosmus* (Figures 17c, 17f) (which has two gonads on the right side); Labs 02, 05, 07, 08, 10, 12, 15, 16 and 19 identified as *Styela clava* (Figures 20b; 25b, 25d) (which has longitudinal stomach grooves).

Fig. 25a. Microcosmus claudicans (RT6625, 67160)



Fig. 25b. Styela clava (P732, 57919) – L



Fig. 25c. *Microcosmus claudicans* (RT6625, 67160) – internal



Fig. 25d. Styela clava (P732, 57919) – internal

Taxonomic and Identification policy considerations highlighted by RT66

An important purpose for the ring test exercises is to highlight areas for further work in identification standardisation and taxonomic research. Several identification problems were highlighted through this exercise, discussed above.

The taxonomic and Identification policy considerations section was added at end of the RT54 bulletin, to include detail for which there was not enough space alongside images. Since RT61, more detailed notes have been provided for each of the families represented in the ring test, as progress towards a Taxonomic Discrimination Protocol (TDP) to standardise future data and help with the interpretation of past and current data. A draft TDP was published last year (Worsfold *et al.*, 2023). For this year's bulletin, discussions are added for all families covered, including those with species named by participants, as well as those with species circulated in RT66, together with those covered in all previous RTs. TDP implications, including recommendations and historical data implications, are also included. Updates to the bibliography document (Worsfold et al., 2020) are due to be produced this year, with combined literature review, RT notes, TDP updates and revision of the UK species lists for selected groups.

Worsfold, T.M, Hall, D.J, & O'Reilly, M. (Ed.), 2020. Bibliography of taxonomic literature for marine and brackish water fauna and flora of the north-east Atlantic. NMBAQC Scheme, 248 pp., May 2020.

Worsfold, T.M., Hall, D.J. & O'Reilly, M. (Ed.), 2023. *Development of standard recording policies for laboratory analysis of north-east Atlantic macrobenthos samples, including a draft Taxonomic Discrimination Protocol (TDP) down to Family level*. Report to the NMBAQC Scheme participants. 48pp, August 2023.

Echinodermata

Echinoderms include many familiar animals but small specimens and certain groups are problematic. In the 1990s, they were often identified using an unpublished guide (Southward, 1972), which was updated as an EBSA guide (Southward & Tyler, 1982), then as an NMBAQC guide (Southward & Picton, 2003), following a workshop in 2003; all are available in archived keys on the NMBAQC website. A field guide (Picton, 1993) was also available. Mortensen (1927 - **M**) was often used for more detailed descriptions, including species from deeper water and distributional limits. The current standard guide (Southward & Campbell, 2006) was produced as a Synopses of the British Fauna (**SBF**) edition.

Mortensen, T., 1927. *Handbook of the echinoderms of the British Isles*. Oxford University Press, Oxford, 471 pp.

Picton, B.E., 1993. A Field Guide to the Shallow-water Echinoderms of the British Isles. Marine Conservation Society, Immel Publishing, 96pp.

Southward, E.C., 1972. *Keys for the Identification of Echinodermata of the British Isles*. Marine Biological Association - Echinoderm Survey, 24 pp., (unpublished).

Southward, E.C. & Campbell, A.C., 2006. *Echinoderms. Keys and notes for the identification of the species.* Synopses of the British Fauna (New Series), No. 56. Published for The Linnean Society of London and The Estuarine and Coastal Sciences Association by Field Studies Council, Shrewsbury, 272 pp.

Southward, E.C., & Picton, B.E., 2003. *Keys for the Identification of Echinodermata of the British Isles, Marine Biological Association - Echinoderm Survey*. NMBAQC Benthic Invertebrate Workshop, Dove Marine Laboratory, Cullercoats, Tynemouth, Nov.2003. 20 pp., (unpublished). NMBAQC scheme RT#66 bulletin Southward, E.C. & Tyler P.A., 1982. *Echinoderms. Estuarine and Brackish-Water Sciences Association - (EBSA) Echinoderm Workshop, Keys and notes*. University of Stirling, 7 - 10 Sep. 1982, 39pp., (unpublished).

<u>Crinoidea</u>

Clark, A.M., 1970. *Echinodermata Crinoidea*. Marine Invertebrates of Scandinavia, 3. Scandinavian University Press, 55 pp.

Antedonidae. All shallow-water Crinoidea in UK waters (the 3 species described in SBF) are antedonids. Others are known from deep water. Adults have always been identified to species, although they often fragment in samples, losing identification features and there has been a tendency to default to *Antedon bifida*. Juveniles are stalked and have previously been recorded as 'Comatulida juv.' at APEM or 'Crinoidea juv.' at some laboratories. The current TDP suggests species identifications for adult antedonids, with separation of juveniles, with identification at family level below 2mm (centrodorsal diameter). *Antedon bifida*, over 5mm, were circulated in 2014 (RT4621), with 8% error.

<u>Asteroidea</u>

Adult starfish are only occasional in macrobenthos samples and usually identified to species. Juveniles are more common and identified to higher levels; these have varied between laboratories, as have the sizes for recognition of juveniles. APEM use a 5mm size limit and leave smaller specimens as 'Asteroidea juv.' in sample data. This is the current TDP suggestion. Identifications are always suggested to be at species level for RT exercises.

Luidiidae. The draft TDP suggests species identifications for adult luidiids, with separation of juveniles (identified at species level) at 10mm, with identification at class level below 5mm, as currently done at APEM. None have yet been circulated but **RT6602** was named *Luidia sarsi* by one lab.

Astropectinidae (RT6602). The draft TDP suggests species identifications for adult astropectinids, with separation of juveniles (identified at species level) at 10mm, with identification at class level below 5mm, as currently done at APEM. **Astropecten irregularis** was first sent in 2024 **(RT6602)**, with 29% error. The specimens were small and would have been recorded as Asteroidea juv. in samples. Mortensen (1927) gives values for the ratios between the length of the arms and the diameter of the disk. The distance from the centre of disk to the point of the arm is designed 'R' and the distance from the centre to the edge of the disk midway between arms, 'r'. In *Asterias rubens*, R = ~4 to 5 times r; in *A. irregularis*, R = 3.5 to 4.5 times r. These measurements are unlikely to apply to juvenile specimens. However, most labs correctly identified them, suggesting some species could be recognised at smaller sizes.

Asteriidae. The draft TDP suggests species identifications for adult asteriids, with separation of juveniles (identified at species level) at 10mm, with identification at class level below 5mm, as currently done at APEM. *Asterias rubens*, over 5mm, were circulated in 1996 (RT0712), without error, 2004 (RT2325), without error and 2012 (RT4306), with 14% error. **RT6602** was named *Asterias rubens* by three labs.

Goniasteridae. The draft TDP suggests species identifications for adult goniasterids, with separation of juveniles (identified at species level) at 10mm, with identification at class level below 5mm, as currently done at APEM. None have yet been circulated but **RT6602** was named Goniasteridae by one lab.

Adult brittlestars are usually identified to species. Juveniles are more common and identified to higher levels; these have varied between laboratories, as have the sizes for recognition of juveniles. APEM use a 3mm disk diameter size limit to define juveniles of all ophiuroids but still identify many to species, with Ophiuridae and Amphiuridae (the most common families in samples) left at family level below 3mm in sample data. This is the current TDP suggestion. Other laboratories leave some as 'Ophiuroidea juv.' and some identify small specimens without separation of juveniles. More discussion may be needed to standardise policies

Ophiuridae (RT6601, RT6608). The draft TDP suggests species identifications for adult ophiurids, with separation of juveniles (identified at family level) at 3mm, as currently done at APEM. *Ophiocten gracilis* was first circulated in 2024 (**RT6601**). Most labs misidentified it (71% error), with the majority (8 labs) naming it as *O. affinis*, the most closely related species included in the Linnean Society guide (Southward & Campbell, 2006). *O. gracilis* was excluded from the standard guide (Southward & Campbell, 2006), as it had been recorded only deeper than 200m and it was included under *O. sericeum* (named by one lab for **RT6601**) in Mortensen (1927) but recognised as a valid species and redescribed by Paterson *et al.* (1982). *Ophiocten affinis* was circulated in 2014 (RT4708), with 60% error. *Ophiura ophiura* was circulated in 2010 (RT3908), with 4% error, and 2014 (RT4625), without error. *Ophiura albida* was circulated in 1994 (RT0120), with 17% error, 1999 (RT1421), with 22% error, and in 2024 (**RT6608**), without error. Two other *Ophiura: O. sarsi*, and *O. robusta*, were used as names for **RT6601** by one lab each. Consistency problems remain with juvenile ophiurids and more discussion may be needed to standardise policies.

Paterson, G.L.J., Tyler, P.A. & Gage, J.D., 1982. The taxonomy and zoogeography of the genus *Ophiocten* (Echinodermata: Ophiuroidea) in the North Atlantic Ocean. *Bulletin of the British Museum (Natural History)*, *Zoology*, 43(3), 109-128.

Amphiuridae (RT6607, RT6609, RT6610, RT6611). The draft TDP suggests species identifications for adult amphiurids, with separation of juveniles (identified at family level) at 3mm, as currently done at APEM. *Amphiura filiformis* was circulated in 1996 (RT0822), with 6% error, 2004 (RT2424), with 7% error, 2019 (RT5709), with 4% error, and 2024 (**RT6609**), without error. **RT6603** was named *Amphiura filiformis* by three labs. *Amphiura chiajei* was circulated in 2006 (RT2716), without error, 2013 (RT4405), with 7% error, 2021 (RT6018), without error, and 2024 (**RT6611**), without error. **RT6607** was named *Amphiura incana* by one lab. **RT6603** was named *Amphiura securigera* by one lab. *Acrocnida brachiata* was circulated in 2000 (RT1610, as *Amphiura*), with 14% error, 2014 (RT4624), with 21% error, and 2024 (**RT6607**) without error. Most labs correctly identified **RT6607**. *Amphipholis squamata* was circulated in 1994 (RT0314), with 13% error, 2000 (RT1609), with 14% error, 2011 (RT4123), with 4% error, 2015 (RT4909), with 20% error, and 2024 (**RT6610**). Most labs correctly identified **RT6610** (6% error), with one leaving it at family level.

Amphilepididae (RT6603). The draft TDP suggests species identifications for amphilepids, with separation of juveniles (identified at species level) at 3mm, as currently done at APEM. *Amphilepis norvegica* was first circulated in 2024 (**RT6603**). Most labs correctly identified it (35% error), although it is excluded from the standard guide (Southward & Campbell, 2006) but included in Mortensen (1927). The most used alternative name was *Amphiura filiformis*; it is likely that most identification differences were due to use of only the standard guide.

Ophiactidae (RT6604). The draft TDP suggests species identifications for ophiactids, with separation of juveniles (identified at species level) at 3mm, as currently done at APEM. **Ophiactis**

balli was first circulated in 2024 (**RT6604**). It was close to the size limit but most labs correctly identified them (6% error). **RT6603** was named *Ophiactis balli* by one lab.

Ophiotrichidae. The draft TDP suggests species identifications for ophiotrichids, with separation of juveniles (identified at species level) at 3mm, as currently done at APEM. *Ophiothrix fragilis* adults were circulated in 2002 (RT2007), with 7% error and 2021 (RT6103), without error. **RT6603** was named *Ophiothrix fragilis* by one lab.

Ophiotomidae. *Ophiocomina nigra* is placed in Ophiocominidae in SBF. The draft TDP suggests species identifications for adult ophiotomids, with separation of juveniles (identified at species level) at 3mm. None have yet been circulated but **RT6604** was named *Ophiocomina nigra* by one lab.

<u>Echinoidea</u>

Adult sea-urchins are only occasional in macrobenthos samples and usually identified to species. Juveniles are more common and identified to higher levels; these have varied between laboratories, as have the sizes for recognition of juveniles. With the exception of Fibulariidae, which only reach a small size, APEM use a 17mm size limit to designate juveniles identified at species, with a 5mm limit below which juveniles are identified at order level. This is the current TDP suggestion in most cases. Other laboratories have used different sizes and different taxonomic levels and further discussion is needed.

Parechinidae (RT6606). The draft TDP suggests species identifications for adult parechinids, with separation of juveniles (identified at species level) at 17mm, with identification at order level (Camarodonta) below 5mm, as currently done at APEM. *Psammechinus miliaris* was circulated in 1998 (RT1225), with 11% error, and in 2024 (**RT6606**). Most labs correctly identified **RT6606** (6% error).

Echinidae. The draft TDP suggests species identifications for adult echinids, with separation of juveniles (identified at species level) at 17mm, with identification at order level (Camarodonta) below 5mm, as currently done at APEM. None have yet been circulated but **RT6605** was named *Gracilechinus elegans* by one lab.

Fibulariidae (RT6606). The draft TDP suggests species identifications for fibulariids, without separation of juveniles, as currently done at APEM. *Echinocyamus pusillus* was circulated in 1994 (RT0210), without error, 2004 (RT2312), without error, 2010 (RT3824), with 3% error, 2011 (RT4105), with 14% error, 2021 (RT6016), without error, and 2024 (**RT6606**), without error.

Loveniidae (RT6614). The draft TDP suggests species identifications for adult loveniids, with separation of juveniles (identified at species level) at 17mm, with identification at order level (Spatangoida) below 10mm, as currently done at APEM. *Echinocardium cordatum* was circulated in 1996 (RT0824), without error, in 2010 (RT3910), with 4% error, in 2016 (RT5105), without error, and in 2024 (**RT6614**), without error. Juvenile sizes may need discussion.

<u>Holothuroidea</u>

Sea cucumbers have usually been identified at lower levels than Holothuroidea, usually species for adults, and are discussed under individual families. A holothurian-specific guide (Madsen & Hansen, 1994 - **MIOS**) is useful for UK species, including several, mainly deeper water species not included in the SBF guide.

Madsen, F.J., & Hansen, B., 1994. *Echinodermata Holothurioidea*. Marine Invertebrates of Scandinavia, 9. Scandinavian University Press, 143 pp.

Cucumariidae (RT6612). Early guides included two Dendrochirotida families in UK waters, Cucumariidae and Psolidae, which were easily distinguishable. Juveniles (defined at 10mm at APEM) were named at family or genus. Following the splitting of Cucumariidae between families, juveniles were named as 'Dendrochirotida juv.', to include Cucumariidae, Sclerodactylidae and Phyllophoridae but not Psolidae (juveniles at genus). Cucumariidae are not usually common in samples. Juveniles are found more often than adults and are usually left at higher taxonomic levels. Older data often lists 'Cucumariidae juv.', as it was possible to recognise them before other families were separated (Sclerodactylidae, Phyllophoridae, below). Since the subdivision of the family, 'Dendrochirotida juv.' has been used, though the change was not immediate at APEM. Some laboratories include them in 'Holothuroidea juv.', probably as they may be difficult to distinguish from members of some of the smaller orders, though these are rare. Adults are usually identified to species, though sizes may vary between laboratories. The current TDP suggests species identifications for adult cucumariids, with separation of juveniles (identified at order level: Dendrochirotida) at 10mm. Ocnus planci was first circulated in 2024 (RT6612). Most labs correctly identified RT6612 (35% error), despite the existence of several similar species. Two other Ocnus are included in Southward & Campbell (2006): O. brunneus and O. lacteus, neither widely recorded. Ocnus brunneus was named for RT6612 by four labs. Aslia lefevrei and Cucumaria frondosa was named for RT6612 by one lab each. Paraleptopentacta elongata was circulated in 2022 (RT6321), with a 14% error.

Synaptidae (RT6613, RT6615). Synaptidae are probably the most widespread holothurians in samples and have usually been identified to species level. Very small specimens and fragments have occasionally been left at higher levels but only rarely and without consistent policy; they have been identified at least to family level at APEM, usually species. The current TDP suggests species identifications for synaptids, without separation of juveniles. *Leptosynapta bergensis* was first circulated in 2024 (**RT6613**). Most labs correctly identified RT6613 (24% error), with four recording as the similar *L. inhaerens. Labidoplax buskii* was circulated in 2015 (RT4925), with 15% error, and 2024 (**RT6615**), without error.

<u>Chordata</u>

The vertebrate classes are excluded from the Benthic 'Invertebrate component' Ring Tests and treated in the 'Fish' component. Appendicularia and Thaliacea are treated under the 'Zooplankton' component.

Cephalochordata

Branchiostomatidae (RT6616). The draft TDP suggests species identifications for branchiostomatids, without separation of juveniles, as currently done at APEM. *Branchiostoma lanceolatum* was circulated in 2011 (RT4114), with a 7% error, 2015 (RT4916), with a 5% error, and 2024 (**RT6616**), without error. Juvenile sizes may need discussion.

<u>Ascidiacea</u>

Sea squirts are widespread in macrobenthos samples, although most are more common on hard substrata, typically surveyed by other methods. Scrape and settlement panels samples are included as macrobenthic and many species are common on mixed substrata and some on soft sediments. Juveniles are present in most survey projects; at APEM, they have been distinguished below 5mm as 'Ascidiacea juv.'. However, some smaller species are identifiable below this size and

there are interstitial species that do not reach 5mm. More discussion may be needed. The standard identification guides have been Berrill (1950) and Millar (1970); also, Hayward & Ryland (1990) included most of the species recorded at the time but many of these were excluded from the more concise update (Hayward & Ryland, 1995), while the most recent edition (Hayward & Ryland, 2017) included some additions, to both previous editions, but excludes many species that had been covered by Hayward & Ryland (1990). Ascidian identification was updated by Brunetti &Mastrototaro (2017) and a seasearch guide (Bowen *et al.*, 2018) gives *in situ* features. However, many species have been newly recorded since, particularly non-natives, and additional resources are necessary; these are considered by family, below.

Berrill, N.J., 1950. *The Tunicata with an account of the British species*. Volume 133. The Ray Society, London, 354 pp.

Bowen, S., Goodwin, C., Kipling, D. & Picton, B., 2018. *Sea squirts and sponges of Britain and Ireland*. Wild Nature Press, Plymouth, for Seasearch, 200pp.

Brunetti, R. & Mastrototaro, F., 2017. Ascidiacea of the European waters. Fauna d'Italia, 51. Calderini, New Business Media, 447pp.

Hayward, P.J. & Ryland, J.S., 1990. *The marine fauna of the British Isles and north-west Europe: Volume 2: Molluscs to chordates*. Clarenden Press, Oxford, 628-996.

Hayward, P.J. & Ryland, J.S., 1995. *Handbook of the marine fauna of the British Isles and north-west Europe*. Clarenden Press, Oxford, 800pp.

Hayward, P.J. & Ryland, J.S. (eds.), 2017. *Handbook of the marine fauna of North-West Europe; Second Edition*. Oxford university press, 785pp.

Millar, R.H., 1970. *British ascidians. Tunicata: Ascidiacea. Keys and notes for the identification of the species.* Synopses of the British Fauna (New Series), No.1. Published for The Linnean Society of London by Academic Press, London and New York, 88 pp.

Didemnidae (RT6619). The draft TDP suggests family level identifications for didemnids, as currently done at APEM. RT6619 (Didemnum vexillum) was the first didemnid to be circulated for the Ring Tests. It is a well-publicized non-native species, which most laboratories (6% error) correctly identified but many problems remain with the family. Other than Didemnum, Millar (1970) includes four species in four genera: Trididemnum tenerum, Leptoclinides faeroensis, Diplosoma listerianum and Lissoclinum argyllense. The MCS guide (Picton, 1985) adds Didemnopsis translucidum (now Trididemnum), Trididemnum cereum, T. delesseriae, Polysyncraton lacazei, P. bilobatum, Diplosoma singulare, D. spongiforme, Lissoclinum perforatum (of which L. argyllense is considered a synonym) and L. weigelei. These same species are listed by Howson & Picton (1997) and MSBIAS. Diplosoma listerianum has been shown to be a species complex (Pérez-Portela et al., 2013) but with only one, cryptogenic, clade in British waters. It was named for RT6622 by one lab. Berrill (1950) describes only two Didemnum: D. gelatinosum and D. maculosum. The Linnean Society guide (Millar, 1970) includes three: D. candidum, D. helgolandicum and D. gelatinosum, all of which are given as synonyms of other species in the Species Directory (Howson & Picton, 1997). D. candidum is included in D. maculosum in the Species Directory but is currently accepted in WoRMS. D. gelatinosum Milne-Edwards, 1841 is Diplosoma listerianum. D. helgolandicum (named for **RT6619** by one lab), though currently accepted on WoRMS, is *D. maculosum*; the type material had larvae with only two adhesive papillae and the identity of the material illustrated by Millar (1970) is unknown (Lambert, 2009). The MCS guide (Picton, 1985) includes four species: D. maculosum, D. coriaceum, D. fulgens and D. lahillei. Howson & Picton (1997) list six species, the four that had been included in Picton (1985) plus D. albidum and D. aspersum. MSBIAS lists eight Didemnum: five of those from the Species Directory (excluding D. aspersum as a synonym of D. NMBAQC scheme RT#66 bulletin

maculosum) and three additional species: *D. candidum, D. pseudofulgens* and *D. vexillum*. The identity of *D. vexillum* as a global non-native was established by Lambert (2009); it had previously been noted at genus level (e.g. Gittenberger, 2007; 2009). Illustrations and identification features for *D. vexillum* are given by Griffith *et al.* (2009) and Tagliapietra *et al.* (2012). Molecular identification has been discussed by Stefaniak et al. (2009), Graham *et al.* (2015), Smith et al. (2012), Matejusona *et al.* (2021), Prentice *et al.* (2021). Control is discussed by Beverage *et al.* (2011). A similar cryptogenic species, *D. pseudovexillum*, was later described from Roscoff (Turon *et al.*, 2020) and could be expected in British waters. It would be useful to improve resolution of didemnid records, due to the importance of some as non-natives but this may be difficult for most; further discussion would be welcome.

Beveridge, C., Cook, E.J., Brunner, L., MacLeod, A., Black, K. Brown, C. & Manson, F.J., 2011. Initial response to the invasive carpet sea squirt, *Didemnum vexillum*, in Scotland. *Scottish Natural Heritage Commissioned Report*, No. 413.

Gittenberger, A., 2007. Recent population expansions of non-native ascidians in The Netherlands. *Journal of Experimental Marine Biology and Ecology*, 342, 122-126.

Gittenberger, A., 2009. Invasive tunicates on Zeeland and Prince Edward Island mussels, and management practices in The Netherlands. *Aquatic Invasions*, 4(1), 279-281.

Graham, J., Collins, C., Lacaze, J.-P., Brown, L. & McCollin, T., 2015. Molecular identification of *Didemnum vexillum* Kott, 1982 from sites around the UK coastline. *BioInvasions Records*, 4(3):,171-177.

Griffith, K. Mowat, S., Holt, R.H.F., Ramsay, K., Bishop, J.D.D., Lambert, G. & Jenkins, S.R., 2009. First records in Great Britain of the invasive colonial ascidian *Didemnum vexillum* Kott, 2002. *Aquatic Invasions*, 4(4), 581-590.

Howson, C.M. & Picton, B.E., (Eds.) 1997. *The species directory of the marine fauna and flora of the British Isles and surrounding seas.* Ulster Museum and the Marine Conservation Society, Belfast and Ross-on-Wye, Ulster Museum Publication No. 276, vi + 508 pp.

Lambert, G., 2009. Adventures of a sea squirt sleuth: unraveling the identity of *Didemnum vexillum*, a global ascidian invader. *Aquatic Invasions*, 4(1), 5-28.

Matejusova, I., Graham, J., Bland, F., Lacaze, J.-P., Herman, G., Brown, L., Dalgarno, E., Bishop, J.D., Kakkonen, J.E., Smith, K.F. & Douglas, A., 2021. Environmental DNA based surveillance for the highly invasive carpet sea squirt *Didemnum vexillum*: a targeted single-species approach. *Frontiers in Marine Science*, 8, 728456.

Pérez-Portela, R., Arranz, V., Rius, M. & Turon, X., 2013. Cryptic speciation or global spread? The case of a cosmopolitan marine invertebrate with limited dispersal capabilities. *Scientific Reports*, 3(3197), 1-10.

Picton, B.E., 1985. *Ascidians of the British Isles: a colour guide*. Marine Conservation Society, Ross-on-Wye. pp 43.

Prentice, M.B., Vye, S.R., Jenkins, S.R., Shaw, P.W. & Ironside, J.E., 2021. Genetic diversity and relatedness in aquaculture and marina populations of the invasive tunicate *Didemnum vexillum* in the British Isles. *Biological Invasions*, 23, 3613-3624.

Smith, K.F., Stefaniak, L., Saito, Y., Gemmill, C.E.C., Cary, S.C. & Fidler, A.E., 2012. Increased intercolony fusion rates are associated with reduced COI haplotype diversity in an invasive colonial ascidian *Didemnum vexillum*. *PLoS ONE*, 7(1), 1-8, e30473.

Stefaniak, L., Lambert, G., Gittenberger, A., Zhang, H., Lin, S. & Whitlatch, R.B., 2009. Genetic conspecificity of the worldwide populations of *Didemnum vexillum* Kott, 2002. *Aquatic Invasions*, 4(1), 29-44.

Tagliapietra, D., Keppel, E., Sigovini, M. & Lambert, G., 2012. First record of the colonial ascidian *Didemnum vexillum* Kott, 2002 in the Mediterranean: Lagoon of Venice (Italy). *BioInvasions Records*, 1(4), 247-254.

Turon, X., Casso, M., Pascual, M. & Viard, F., 2020. Looks can be deceiving: *Didemnum pseudovexillum* sp. nov. (Ascidiacea) in European harbours. *Marine Biodiversity*, 50(48), 1-14.

Holozoidae. The draft TDP suggests family identifications for holozoids over 5mm, recorded as presence / absence, with juveniles left at Class (Ascidiaceae), as currently done at APEM. None have yet been circulated but *Distaplia rosea* was named for **RT6622** by one lab.

Polyclinidae. The draft TDP suggests family identifications for polyclinids over 5mm, recorded as presence / absence, with juveniles left at Class (Ascidiaceae), as currently done at APEM. None have yet been circulated but *Aplidium nordmanni* was named for **RT6622** by one lab and *Aplidium urgorri* was named for **RT6622** by one lab.

Ascidiidae (RT6623). The draft TDP suggests species identifications for ascidiids over 5mm, with juveniles left at Class (Ascidiaceae), as currently done at APEM. Ascidiella aspersa was circulated in 1994 (RT0218), with 17% error, 2020 (RT5820), with 47% error and 2021 (RT6024), with 12% error. It was named for RT6617 by one lab and for RT6623 by six labs. Ascidiella scabra was circulated for the first time in 2024 (RT6623); it is common both on artificial and mixed substrata. Most labs misidentified RT6623 (65% error), most identifying as the similar A. aspersa. Some of the features given to separate Ascidiella species in guides may be unreliable. Relative positions of siphons and siphon pigment are useful for estimates with large numbers of specimens but do not guarantee correct identifications for all individuals. Dorsal tubercle shape is variable in both species. Both Berrill (1950) and Millar (1970) state that one of the few consistent differences between them is the number of oral tentacles relative to the number of branchial bars: tentacles more numerous than bars in in A. scabra. A. scabra was named for RT6618 and for RT6624 by one lab each. Ascidia mentula was named for RT6623 by two labs. The lack of papillae on the branchial bars of Ascidiella (Millar, 1970) is open to misinterpretation and, ufortunately, we have not found good illustrations in the literature; it is the secondary papillae, where the longitudinal and transverse vessels meet, that are lacking in Ascidiella, not the papillae that give rise to the longitudinal vessels and are present in all Ascidiidae holding up the branchial bars; papillae in Ascidiella tend to be confined to beneath the branchial bars and don't protrude much above; in Ascidia they are much more pronounced and frequently occur between the junctions (Berrill, 1950). Both Ascidiella species are common fouling animals and cryptogenic (Nishikawa et al., 2014; Nydam et al., 2022). Ascidia obliqua was named for RT6623 by two labs and for RT6618 by one lab. Phallusia mammillata was named for RT6617 and for RT6624 by one lab each. Although confusion remains in the identification of ascidiids, the policy may need review only for standardisation of juvenile sizes.

Nishikawa, T., Oohara, I., Saitoh, K., Shigenobu, Y., Hasegawa, N., Kanamori, M., Baba, K., Turon, X. & Bishop, J.D.D., 2014. Molecular and morphological discrimination between an invasive ascidian *Ascidiella aspersa* and its congener *A. scabra* (Urochordata: Ascidiacea). *Zoological Science*, 31(3), 180-185.

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Nydam, M.L., Nichols, C.L. & Lambert, G., 2022. First record of the ascidian Ascidiella aspersa (Müller, 1776) in southern California. *BioInvasions Records*, 11(2), 416-427.

Cionidae. The draft TDP suggests species identifications for cionids over 5mm, with juveniles left at Class (Ascidiaceae), as currently done at APEM. A second *Ciona* species has been found as a non-native (Brunetti et al. (2015). *Ciona intestinalis* was circulated in 2018 (RT5501), with 30% error.

Brunetti, R., Gissi, C., Pennati, R., Caicci, F. & Manni, L., 2015. Morphological evidence that the molecularly determined *Ciona intestinalis* type A and type B are different species: *Ciona robusta* and *Ciona intestinalis*. *Journal of Zoological Systematics and Evolutionary Research*, 53(3), 186-193.

Corellidae. The draft TDP accidentally excluded Corellidae; we suggest species identifications for corellids over 5mm, with juveniles left at Class (Ascidiaceae), as currently done at APEM. *Corella eumyota* is a recent non-native arrival (Collin et al., 2010; Lambert, 2004). None have yet been circulated but *Corella eumyota* was named for **RT6617** by one lab and *Corella parallelogramma* was named for **RT6623** by one lab.

Collin, S.B., Oakley, J.A., Sewell, J. & Bishop, J.D.D., 2010. Widespread occurrence of the nonindigenous ascidian *Corella eumyota* Traustedt, 1882 on the shores of Plymouth Sound and Estuaries Special Area of Conservation, UK. *Aquatic Invasions*, 5(2), 175-179.

Lambert, G., 2004. The south temperate and Antarctic ascidian *Corella eumyota* reported in two harbours in north-western France. *Journal of the Marine Biological Association of the United Kingdom*, 84(1), 239-242.

Pyuridae (RT6625). The draft TDP suggests species identifications for pyurids over 5mm, with juveniles left at Class (Ascidiaceae), as currently done at APEM. *Microcosmus claudicans* was circulated for the first time in 2024 (**RT6625**); it is occasional in mixed substrata. Most labs misidentified **RT6625** (65% error), most identifying as *Styela clava*, probably due to the apparent stalk on the circulated specimens. The stalks were due to their having been attached to subsurface particles and not the same structure as *Styela clava* stalks. *Pyura microcosmos* was named for **RT6620** and **RT6625** by two labs each and for **RT6621** and **RT6617** by one lab each. *Pyura tessellata* was named for **RT6620** by one lab.

Styelidae (RT6617, RT6620, RT6621, RT6622, RT6624). The draft TDP suggests species identifications for styelids over 5mm, with juveniles left at Class (Ascidiaceae), as currently done at APEM. Styelids include both solitary and colonial species, which should be counted or recorded as present, respectively. *Dendrodoa grossularia* may be abundant on stones and shells in mixed substratum samples and has been circulated twice. For RT1016 (1997), percentage error was 32%; in 2024, most labs identified **RT6624** correctly (24% error) but it was named for **RT6618** and for **RT6621** by one lab each. Four styelids were circulated for the first time in 2024 (RT66). *Asterocarpa humilis* is a non-native (southern hemisphere) species that was first reported from Brittany in 2005 and from English marinas in 2009 (Bishop *et al.*, 2013). It was first circulated in 2024 (**RT6617**) and most labs misidentified it (59% error), with eight alternative names, most used by only one lab each. It was named for **RT6621** by two labs. *Styela canopus* is a potential species complex and cryptogenic (Corrêa de Barros & Moreira da Rocha, 2022), often found on pontoons. in It was first circulated in 2024 (**RT6620**) and most labs misidentified it (76% error), with five alternative names. It was named for **RT6617** by two labs and for **RT6621** by one lab each. *Styela coriacea* was named for **RT6617**, **RT6620** and for **RT6621** by one lab each. *Styela clava*

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was named for **RT6625** by nine labs, for **RT6620** by five labs and for **RT6621** by two labs. *Polycarpa pomaria* may be common on stones and shells in mixed substratum samples. It was first circulated in 2024 (**RT6621**) and most labs misidentified it (53% error), with seven alternative names, most used by only one lab each. It was named for **RT6620** by four labs and for **RT6617** by four labs. *Polycarpa fibrosa* was named for **RT6621** by three labs. *Stolonica socialis* was named for **RT6624** by one lab. The colonial, non-native (Japan) *Botrylloides violaceus* was recorded from several marinas on the south coast of England in 2004 but is likely to have been established before then (Arenas et al., 2006). It was first circulated in 2024 (**RT6622**) and many labs misidentified it (41% error), with five alternative names, most used by only one lab each. *Botrylloides leachii* was named for **RT6622** by three labs. TDP updates may be needed to account for colonial and cryptic species.

Arenas, F., Bishop, J.D.D., Carlton, J.T., Dyrynda, P.J., Farnham, W.F., Gonzalez, D.J., Jacobs, M.W., Lambert, C., Lambert, G., Nielsen, S.E., Pederson, J.A., Porter, J.S., Ward, S. & Wood, C.A., 2006. Alien species and other notable records from a rapid assessment survey of marinas on the south coast of England. *Journal of the Marine Biological Association of the United Kingdom*, 86 (6), 1329-1338.

Bishop, J.D.D., Roby, C., Yunnie, A.L.E., Wood, C.A., Lévêque, L., Turon, X. & Viard, F., 2013. The Southern Hemisphere ascidian *Asterocarpa humilis* is unrecognised but widely established in NW France and Great Britain. *Biological Invasions*, 15, 253-260.

Corrêa de Barros, R. & Moreira da Rocha, R., 2022. Genetic analyses reveal cryptic diversity in the widely distributed *Styela canopus* (Ascidiacea: Styelidae). *Invertebrate Systematics*, 35, 298-311.

Molgulidae (RT6618). The draft TDP flags varied options (more work required) for molgulids over 5mm, with juveniles left at Class (Ascidiaceae), as currently done at APEM. *Molgula manhattensis* was circulated in 1996 (RT0717), with a percentage error of 61 and in 2021 (RT6014, as an aggregate to include *M. socialis*), with a percentage error of 18. It was named for **RT6617** and for **RT6618** by one lab each. *Molgula complanata* was first circulated in 2024 (**RT6618**), with 41% error. There were five alternative names but many identifications were correct, for a difficult species, and only two generic errors. *Molgula citrina* was named for **RT6618** by three labs. The policy may need review for standardisation of juvenile sizes, to allow greater consistency and to account for cryptic species.

Acknowledgements

We are grateful to Sabine Stöhr (Swedish Museum of Natural History) for advice on identification of *Ophiocten* species. We would also like to thank all participants who provided feedback following issue of interim results.

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Ring Test Specimen Return Instructions

Please return all ring test specimens by <u>30th August 2024.</u> These are reference collection specimens and must be returned to our museum. Your laboratory will be ineligible for future ring tests if specimens are not returned.

Return address: David Hall, APEM Ltd., 7a Diamond Centre, Works Road, Letchworth, Hertfordshire SG6 1LW, UK