Appendix 1

Taxon lists

Table 1**:** Taxon sampling: taxa on which molecular analysis was carried out during that project. (Both samples which did and did not amplify successfully are included)

Specimin code	Family	Genus	Species	adult/ larvae	Host association	Location(1)	Location(2)	Year collected	28s 300F ECD2	ITS2
TS 09/272	Acrobothriidae	unknown	unknown	larvae	Scorpion fish intestine	Petunia, Svalbard	Sweden	2009		
AR12	Acrobothriidae	*Spathebothrium*	sp.	adult	Scorpion fish intestine	Petunia, anchorage, sand Svalbard	Sweden	2008	Y	Y
TS 09/271	Acrobothriidae	*Diplocotyle*	sp.	adult	Twohorn sculpin intestine	Petunia, anchorage, sand Svalbard	Sweden	2008	Y	Y
TS 09/275	Acrobothriidae	*Diplocotyle*	sp.	adult	Scorpion fish intestine	Lyskteneset Svalbard	Sweden	2009	Y	
TS 09/277	Acrobothriidae	*Diplocotyle*	sp.	adult	Scorpion fish intestine	Scottenia Svalbard	Sweden	2009		
TS 09/279	Acrobothriidae	*Diplocotyle*	sp.	adult	Scorpion fish intestine	Petunia, Svalbard	Sweden	2009	Y	Y
TS 09/280	Acrobothriidae	*Diplocotyle*	sp.	adult	Twohorn sculpin intestine	Petunia, Svalbard	Sweden	2009	Y	
TS 09/282	Acrobothriidae	*Diplocotyle*	sp.	adult	Scorpion fish intestine	Scottenia Svalbard	Sweden	2009	Y	
TS 09/283	Acrobothriidae	*Diplocotyle*	sp.	adult	Scorpion fish intestine	Scottenia Svalbard	Sweden	2009	Y	
TS 09/284	Acrobothriidae	*Diplocotyle*	sp.	adult	Scorpion fish intestine	Petunia, Svalbard	Sweden	2009	Y	Y

Got 221	Acrobothriidae	*Bothrimonus*	*fallax*	adult	Sturgeon? Intestine	Caspian Sea, Karmar-Kulali, Tavush Province	Armenia	2010		
Stoc 88/20	Acrobothriidae	*Bothrimonus*	*fallax*	adult	Starry sturgeon intestine	Caspian Sea, Karmar-Kulali, Tavush Province	Armenia	2010		
TS 04/10	Acrobothriidae	*Bothrimonus*	fallax		Fringebarbel sturgeon intestine	Caspian Sea	Russia			
Stoc 382	Acrobothriidae	*Cyathocephalus*	*truncatus*	adult	Alaskan Fourhorn sculpin intestine	Lake Vanern	Sweden			
Stoc 385	Acrobothriidae	*Cyathocephalus*	*truncatus*	adult	Freshwater shrimp intestine	Lake Hemsjon	Sweden			
TS 00/5	Acrobothriidae	*Cyathocephalus*	*truncatus*	adult	Sea trout intestine	Brenta River, Farrara	Italy	1998		
TS 04/136	Acrobothriidae	*Cyathocephalus*	*truncatus*	adult	Common whitefish intestine	Lake Segozero Karelia	Russia	2004	Y	Y
TS 06/9	Acrobothriidae	*Cyathocephalus*	*truncatus*	adult	Unknown sp. Intestine	Lake Segozero Karelia	Russia	2004	Y	Y
Got 481a	Acrobothriidae	*Didymobothrium*	*rudolphii*	adult	Sand sole intestine	Banyuls-sur-mer	France			
Got 481b	Acrobothriidae	*Didymobothrium*	*rudolphii*	adult	Sand sole intestine	Banyuls-sur-mer	France			
AR3	Acrobothriidae	*Diplocotyle*	*sp.*	adult	Scorpionfish sp. Intestine	Petuniabucta Svalbard	Sweden	2008	Y	Y
Got 552	Acrobothriidae	*Diplocotyle*	*nylandicus*	adult	Flounder (but diff latin to others?) intestine	Baltic sea				
Stoc 338a	Acrobothriidae	*Diplocotyle*	*nylandicus*	adult	Flounder intestine	Lofo	Finland	2010		
Stoc 338b	Acrobothriidae	*Diplocotyle*	*nylandicus*	adult	Flounder intestine	Lofo	Finland	2010		
TS 01/1	Acrobothriidae	*Diplocotyle*	*olrikii*	adult	Brook trout intestine	San Pierro (Miquelon-France) Near New Foundland	Canada	2000		

AR23	Spathebothriidae	*Spathebothrium*	*simplex*	adult	Gelatinous snailfish intestine	Petunia, German camp, Svalbard	Sweden	2008	Y	Y
BFAX	Acrobothriidae	*Bothrimonous*	*fallax*	adult	Fringebarbel sturgeon ex	Caspian Sea	Russia			
Gcot 6	Gyrocotyliidae	*Gyrocotyle*	*sp.*	adult	Pacific longnose Chimaera spiral intestine	Goban Spur, North Atlantic		2001		Y
Gcot 7	Gyrocotyliidae	*Gyrocotyle*	*sp.*	adult	Large eyed rabbitfish spiral intestine	Porcupine bight, North Atlantic	United kingdom	2002		Y
Gig	Amphilinidae	*Gigantolina*	*magna*	adult	Painted sweetlips body cavity	Coral sea, heron Island, Queensland	Australia	1998		Y
Ampf	Amphilinidae	*Amphilina*	*foliacea*	adult	Sterlet body cavity	Lower Danube river	Bulgaria	2005		Y
Ael	Amphilinidae	*Austramphalina*	*elongata*	adult	Eastern long necked turtle	Amirdale new south wales	Australia			Y

Table 2: Taxon sampling: Taxa for which existing sequences (from other studies) were used within this project.

Specimin code	Family	Genus	Species	adult/ larvae	Host association	Location(1)	Location(2)	Year collected	28s 300F ECD2	ITS2
Cyt	Acrobothriidae	*Cyathocephalus*	truncatus	adult	Sea trout intestine	Areuse river	Switzerland	1999	Y	Y
Didb10	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	Central portuguese coast	Portugal	2003	Y	Y
Didb11	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	Central portuguese coast	Portugal	2003	Y	Y
Didb12	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	South Portuguese coast	Portugal	2003	Y	Y
Dibd13	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	South Portuguese coast	Portugal	2003	Y	Y
Didb15	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	South Portuguese coast	Portugal	2003	Y	Y
Didb4	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	North Portuguese coast	Portugal	2003	Y	Y
Didb5	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	North Portuguese coast	Portugal	2003	Y	Y
Didb6	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	North Portuguese coast	Portugal	2003	Y	Y
Didb7	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	North Portuguese coast	Portugal	2003	Y	Y
Didb8	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	Central portuguese coast	Portugal	2003	Y	Y
Dibd9	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	Central portuguese coast	Portugal	2003	Y	Y
JmCA	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	Central portuguese coast	Portugal	2003	Y	Y

JmCS	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	Central portuguese coast	Portugal	2003		Y
JmCW	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	Central portuguese coast	Portugal	2003	Y	Y
JmNA	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	North Portuguese coast	Portugal	2003	Y	Y
JmNS	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	North Portuguese coast	Portugal	2003	Y	Y
JMNSp	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	North Portuguese coast	Portugal	2003	Y	Y
JmNW	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	North Portuguese coast	Portugal	2003	Y	Y
JmCSP	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	Central portuguese coast	Portugal	2003	Y	
JmSS	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	South Portuguese coast	Portugal		Y	
JmSA	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	South Portuguese coast	Portugal	2003	Y	Y
JmSSp	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	South Portuguese coast	Portugal	2003	Y	Y
JmSW	Acrobothriidae	*Didymobothrium*	rudolphii	adult	Sand sole intestine	South Portuguese coast	Portugal	2003	Y	Y
Bothm (diplo)	Acrobothriidae	*Diplocotyle*	olrikii	adult	Freshwater shrimp haemocoel	North sea intertidal beach area infront of castle, St Andrews	Scotland	2004	Y	Y
SPS	Spathebothriidae	*Spathebothrium*	simplex	adult	Gelatinous snailfish intestine	Atlantic Ocean, Rye beach, New hampshire	USA	1997	Y	Y
Gcot 7	Gyrocotyliidae	*Gyrocotyle*	*sp.*	adult	Large eyed rabbitfish spiral intestine	Porcupine bight, North Atlantic	United kingdom	2002	Y	

Gig	Amphilinidae	*Gigantolina*	*magna*	adult	Painted sweetlips body cavity	Coral sea, heron Island, Queensland	Australia	1998	Y	
Ampf	Amphilinidae	*Amphilina*	*foliacea*	adult	Sterlet body cavity	Lower Danube river	Bulgaria	2005	Y	
Ael	Amphilinidae	*Austramphalina*	*elongata*	adult	Eastern long necked turtle	Amirdale new south wales	Australia		Y	

Appendix2

PCR and sequencing primers
Thermocycler conditions for primer combinations

Table 1: PCR primers used for amplification of 28S, ITS2 and COI regions

Target region	Primer forward	Primer reverse	Fragment size (bp)	Source
28s	**LSU5** 5' TAG GTC GAC CCC CTG AAY TTA AGC 3'	**1200R** 5' GCA TAG TTC ACC ATC TTT CGG 3'	1400	Olson et al 2001
COI	**Plat_diploCOXIF** 5' CGT TTR AAT TAT ACG GAT CC 3'	**Plat_diploCOXIR** 5' AGC ATA GTA ATM GCA GCA GC 3'	500	Moszczynska et al 2009
	FPI 5' TGT TTA GAG GTA GGA AGG GTG 3'	**RPI** 5' ACC CGA AGA GGC AAA CCA CAC 3'		Laboratory colleague
	MplatCOXIdF 5' TGTAAAACGACGGCCAGTTTWCITTRGATCATAGG 3'	**MplatCOXIdR** **5'** CAG GAA ACA GCT TAG ACT GAA AYA AYA IIG GAT CIC CAC C 3'	650	Moszczynska et al 2009
	Cyclo_cox1Fa 5 'CAR CAT ATG TTT TGR TTT TTT GG 3'	**Cyclo_16SRc** 5' AAT AGA TAA GAA CCG ACC TGG C 3'	1900	Littlewood et al 2008
	LCO1490 5' GGT CAA CAA ATC ATA AAG ATA TTG G 3'	**HCO2198** 5' TTA ACT TCA GGG TGA CCA AAA AAT CA 3'	700	Laboratory colleague
ITS2	**ITS2.3S** 5' GGT ACC GGT GGA TCA CGT GGC TAG TG 3'	**ITS2.2** 5' CCT GGT TAG TTT CTT TTC CTC CGC 3'	600	Olson et al 2001

Table 2: Thermocycling conditions for 28S, ITS2 and COI primers detailed in table 1.

Primer combination	Initial Denaturation	Number of cycles	Denaturation	Annealing	Extension	Final extension
LSU5-1200R	95°C 5mins	40	95°C 30secs	52°C 30secs	72°c 1min 30secs	72°c 7mins
Plat_diploCOXIF-Plat_diploCOXIR	94°C 2mins	35	94°C 30secs	50°C 30secs	72°c 1min	72°c 10mins
FPI-RPI	94°C 3mins	40	94°C 30secs	55°C 30secs	72°c 3min	72°c 7mins
MplatCOXIdF-MplatCOXIdR	94°C 2mins	35	94°C 30secs	50°C 30secs	72°c 1min	72°c 10mins
Cyclo_cox1IFA - Cyclo_16SRc	94°C 3mins	40	94°C 30secs	52°C 30secs	72°c 3min	72°c 7mins
LCO 1440-HCO 2198	95°C 5mins	43	94°C 45secs	40/45°C 45secs	72°c 2min	72°c 10mins
ITS2.3S-ITS2.2	95°C 3mins	44	94°C 30secs	56°C 30secs	72°c 1min	72°c 7mins

Table 3: Additional sequencing primers for 28S and COI regions

Region	PCR primers	Additional sequencing primers (F then R)
28S	LSU5-1200R	**300F** 5'CAA GTA CCG TGA GGG AAA CTT3' **ECD2** 5' CTT GGT CCG TGT TTC AAG ACG GG3'
COI	Cyclo_coxIFa-Cyclo_16SRc	**Cyclo_coxIFa** 5'CAR CAT ATG TTT TGR TTT TTT GG 3' **Cyclo_coxIRb** 5'CCT AAY GAC ATA ACA TAA TGR AAA TG 5'

Table 4: Nested PCR protocol for 18S and 28S

Gene region	Initial PCR	Nested PCR primers	Fragment size (bps)
18S			
	WA -1270R	WA-CESTV4R	820
		600F-1270R	770
		600F-CESTV4R	300
	930F-WB	930F-1270R	340
		1270F-1600R	330
		1600F-WB	400
28S			
	LSU5-1200R	LSU5-300R	350
		300F-ECD2	700
		900F-1200R	450
		LSU5-ECD2	1100
	LSU5-ECD2	LSU5-400R	450

Table 5: Initial and nested PCR primers for 18S and 28S

Target region	Forward primers	Reverse primers
18S	**WA** 5' GCG AAT GGC TCA TTA AAT CAG 3'	**1270R** 5' CCG TCA ATT CCT TTA AGT 3'
	600F 5' GGT GCC AGC MCG GGC G 3'	**CESTV4R** 5' CAA AGT AAA CGT GCC ATC 3'
	930F 5' GCA TGG AAT AAT GGA ATA GG 3'	**WB** 5' CTT GTT ACG ACT TTT ACT TCC 3'
	1270F 5' ACT TAA AGG AAT AGA CGG 3'	**1600R** 5' GGG CAT CAC AGA CCT G 3'
	1600F 5' CAG GTC TGT GAT GCC C 3'	
28S	**LSU5** 5' TAG GTC GAC CCG CTG AAY TTA AGC 3'	**1200R** 5' GCA TAG TTC ACC ATC TTT CGG 3'
	300F 5' CAA GTA CCG TGA GGG AAA GTT G 3'	**300R** 5' CAA CTT TCC CTC ACG GTA CT T G 3'
	900F 5' CCG TCT TGA AAC ACG GAC CAA C 3'	**ECD2** 5' CTT GGT CCG TGT TTC AAG ACG GG3'
		400R 5' GCA GCT TGA CTA CAC CCG 3'

Table 6: Spathebothrrid specific PCR primers for 18S and 28S

Target region	Primer forward	Primer reverse	Fragment size	Optimised annealing temperature (^{o}C)
18S	**Spath18s_430F** 5' TGA ACG AGG CTC CG TAA T 3'	**Spath18s_600R** 5' TAG GCA GGC AAC ART GAT AC 3'	175	55
	Spath18s_600F 5' GTA TCA YTG TTG CCT GCC TAW YC 3'	**Spath18s_680R** 5' CCA AAC CCA CTC ARC ATG C 3'	100	59
	Spath18s_680F 5' GCA TGY TGA GTG GGT TTG G 3'	**Spath18s_1350R** 5' CAC CAC CAA CCA CTA AAT CAA GAA 3'	700	55
	Spath18s_1350F 5' CTT GAT TTA GTG GTT GGT G 3'	**Spath18s_1470R** 5' CAG TCT CAC TGA AAC GCC ACC 3'	150	N/A
	Spath18s_1470F 5' GGT GGC GTT TCA GTG AGA CTG 3'	**Spath18s_1600R** 5' GAA CCC GGA AGT AAA CGC T 3'	150	58
28S	**Spath28s_460F** 5' GGA TTC AGY CAG TCA GGA TTK TG 3'	**Spath28s_600R** 5' GAC GAA TAG CTT GCT CCG 3'	150	55
	Spath28s_620F 5' GTC TAG AGG ACT GCC TGG CTG 3'	**Spath28s_700R** 5' GCC RAC ATY AGA TGC CGA 3'	120	55
	Spath28s_700F 5' GTC GGC ATC TRA TGT YGG C 3'	**Spath28s_780R** 5' CCA GYG THC GAT ARC GTG CAA 3'	100	55
	Spath28s_780F 5' TTG CAC GYT ATC GDA CRC TGG 3'	**Spath28s_950R** 5' AAC CCA AAC ACC GGC AGG CT 3'	195	55
	Spath28s_950F 5' CAG CCT GCC GGT GTT TGG 3'	**Spath28s_1100R** 5' CAA CAA CTC CAA CCG AGC 3'	90	59