APPENDIX 1 DETAILS OF KELP SPECIMENS – ORDER LAMINARIALES SHOWN BY FAMILY

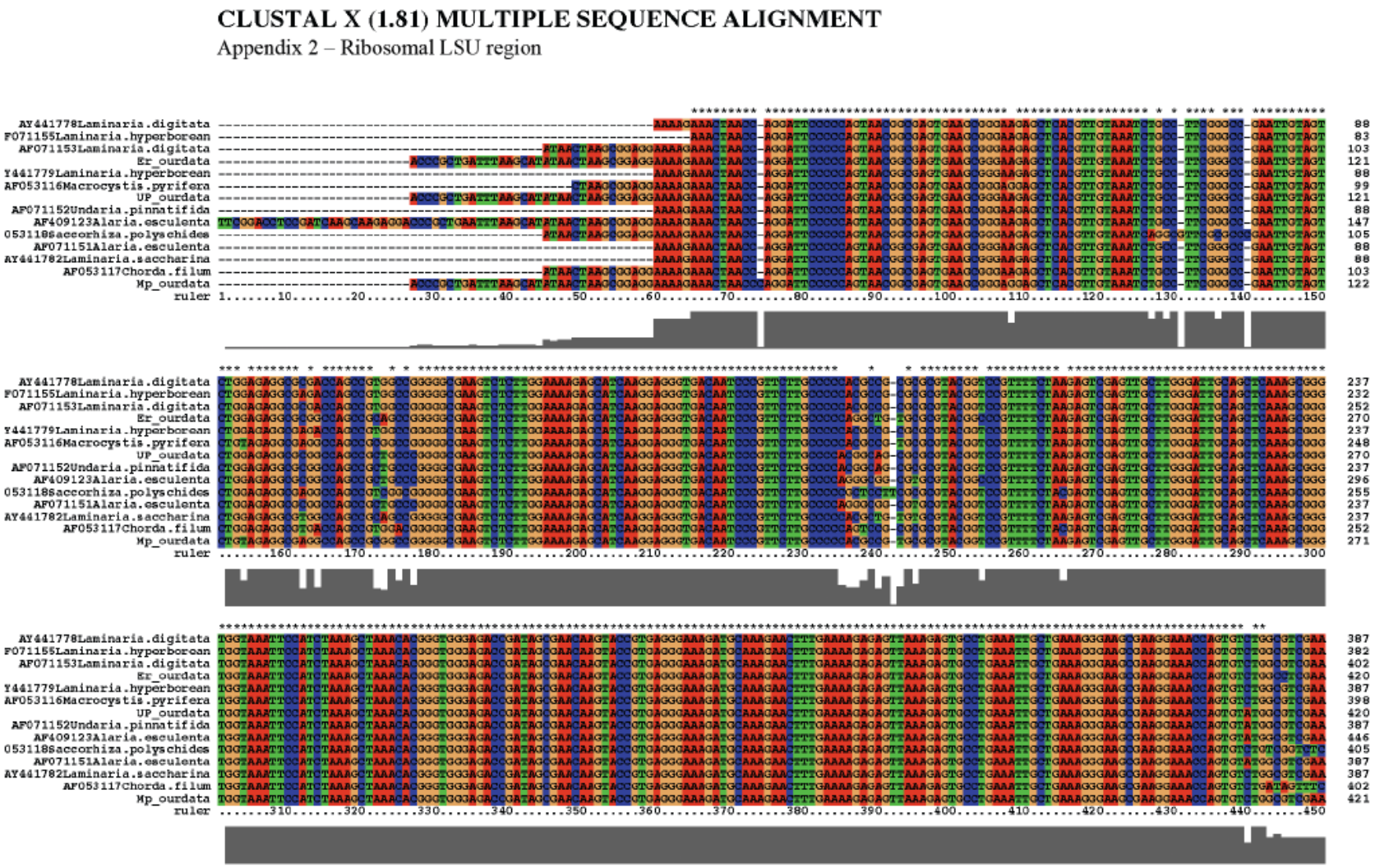
Species	Location	Sample code/s	n	Sample type	Collected by
Family Alariaceae					
Undaria pinnatifida	Williamstown, Port Phillip Bay, Victoria, Australia	UP7, UP8, UP9	3	Frozen	John Lewis
U. pinnatifida	Flinders Jetty, Victoria, Australia	1- GWS000967	1	DNA	Gary W. Saunders
U. pinnatifida	Bloodstone Bay, Maria Island, Tasmania, Australia	UP10, UP11, UP12	3	Silica dried	Britta Schaffelke
U. pinnatifida	Georges Bay, St. Helens, Tasmania, Australia	UP13, UP14, UP15	3	Silica dried	Britta Schaffelke
U. pinnatifida	Tinderbox, Tasmania, Australia	UP16, UP17, UP18	3	Silica dried	Britta Schaffelke
U. pinnatifida	Triabunna, Tasmania, Australia	UP22, UP23, UP24	3	Silica dried	Britta Schaffelke
U. pinnatifida	Triabunna, Lords Bluff, Tasmania, Australia	UP25, UP26, UP27	3	Silica dried	Britta Schaffelke
U. pinnatifida	Waibs Beach, Bicheno, Tasmania, Australia	UP28, UP29, UP30	3	Silica dried	Britta Schaffelke
U. pinnatifida	Tinderbox, Tasmania, Australia	UP1 – UP6	6	Frozen	Cath Sliwa
U. pinnatifida	Mar Piccolo, Taranto, Italy (Ionian Sea)	UP31, UP32, UP33	3	Silica dried	Antonella Petrocelli
U. pinnatifida	Neeltje Jans, Province of Zeeland, The Netherlands	UP34, UP35, UP36	3	Silica dried	Herre Stegenga
U. pinnatifida	L'Etang de Thau, France	2-UF01ME	1	DNA	Gary W. Saunders
U. pinnatifida	Nelson, New Zealand	UP37, UP38, UP39	3	Silica dried	Ashely Coutts
U. pinnatifida	Wellington, New Zealand	UP40, UP41, UP42	3	Silica dried	Britta Schaffelke
U. pinnatifida	Carey's Bay, Otago harbour, New Zealand	UP 43, UP44, UP45	3	Silica dried	Britta Schaffelke
U. pinnatifida	Oura Bay, Shimoda, Shizuoka Prefecture, Japan	UP 46, UP47, UP48	3	Oven dried at 50ºC	Alecia Bellgrove
U. pinnatifida	Sakamoto, Shizugawa, Miyagi Prefecture, Japan	UP 49, UP50, UP51	3	Oven dried at 50ºC	Alecia Bellgrove

Species	Location	Sample code/s	n	Sample type	Collected by
U. pinnatifida	Padori-4, Taean, Chungnam, Korea	UP52	1	Silica dried	Sung Min Boo
U. pinnatifida	Anin-4,Gangreung, Gangwon, Korea	UP53	1	Silica dried	Sung Min Boo
U. pinnatifida	Gabaeri-1,Geojedo,Gyeongnam, Korea	UP54	1	Silica dried	Sung Min Boo
U. pinnatifida	Portsmouth Harbour, United Kingdom	UP55, UP56, UP57	3	Oven dried at 40ºC	Paul Farrell
U. pinnatifida	Nuevo Gulf, Argentina	UP58, UP59, UP60	3	Silica dried	Graciela Casas
U. undarioides	Wakayama-3, Japan	Uu1	1	Silica dried	Sung Min Boo
Undariella peterseniana	Udo-1,Jeju, Korea	Upet1	1	Silica dried	Sung Min Boo
Alaria esculenta	An Tra Beag, near Spiddal, County Galway, Ireland	Ae1, Ae2, Ae3	3	Silica dried	Stefan Kraan
A. marginata	Seal Rock, Oregon, USA	31-AM01SR, 32-AM02SR	2	DNA	Gary W. Saunders
A. marginata	Kirby PT., Bamfield, British Columbia, Canada	6 - AM01KP	1	DNA	Gary W. Saunders
A. nana	Garapata Beach, Hopkins Marine Center, California, USA	33 - AN06GB	1	DNA	Gary W. Saunders
Ecklonia radiata	Williamstown, Port Phillip Bay, Victoria, Australia	Er4, Er5, Er6	3	Frozen	John Lewis
E. radiata	Tinderbox Beach, Tasmania, Australia	Er1, Er2, Er3	3	Frozen	Felicity McEnnulty
E. radiata	Bridport, Tasmania, Australia	Er7, Er8, Er9	3	Frozen	Felicity McEnnulty
E. radiata	Whitefords Reef, near Perth, Western Australia, Australia	3- ECO1WR	1	DNA	Gary W. Saunders
Eisenia arborea	Kirby PT., Bamfield, British Columbia, Canada	10-EA01KP, 11-EA02KP	2	DNA	Gary W. Saunders
Egregia menziesii	Botany Beach, Port Renfrew, British Columbia, Canada	9 - EM02BA	1	DNA	Gary W. Saunders
E. menziesii	Boiler Bay, Oregon, USA	35-EM03BB,	1	DNA	Gary W. Saunders
E. menziesii	Boiler Bay, Oregon, USA	36-EM04BB, 37-EM05BB	2	DNA	Gary W. Saunders
Pterygophora californica	Cape Beale, Bamfield, British Columbia, Canada	27-PC01CB, 28-PC02CB	2	DNA	Gary W. Saunders
Species	Location	Sample code/s	n	Sample type	Collected by

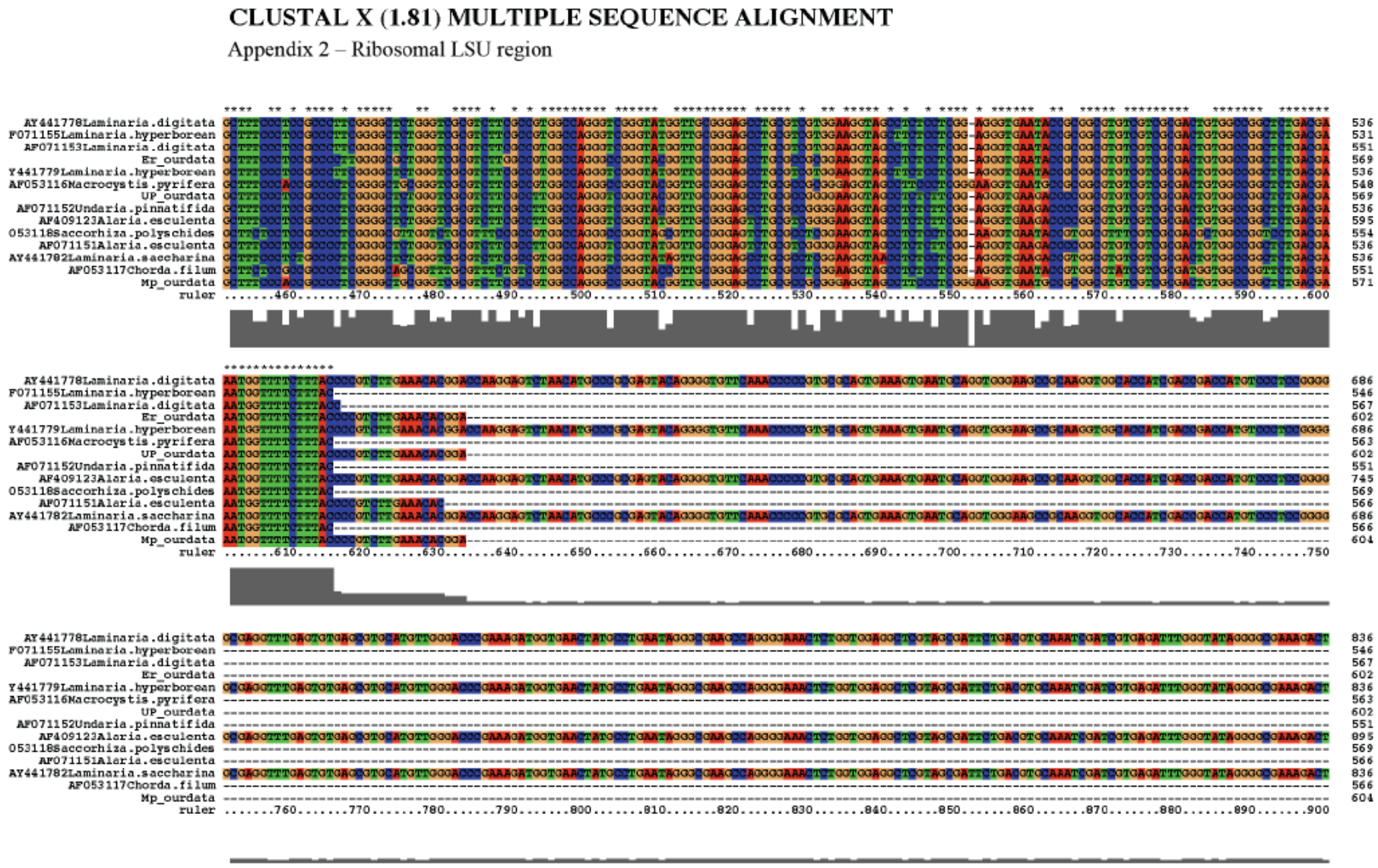
Family Laminariacea					
Agarum clathratum	Grand Manan, New Brunswick, Canada.	5 - CL000602	1	DNA	Gary W. Saunders
Costaria costata	Whiffen Spit, British Columbia, Canada	7 - COST	1	DNA	Gary W. Saunders
Cymathere triplicata	Whiffen Spit, British Columbia, Canada	8 - CYMA	1	DNA	Gary W. Saunders
Hedophyllum sessile	Cape Beale, Bamfield, British Columbia, Canada	12 - CL001501	1	DNA	Gary W. Saunders
Kjellmaniella gyrata	Culture: L. Druehl, Bamfield, British Columbia, Canada	13 - Kgcul	1	DNA	Gary W. Saunders
Laminaria digitata	Lepreau, New Brunswick, Canada	14 - CL030103	1	DNA	Gary W. Saunders
L. saccharina	Culture: L. Druehl, Bamfield, British Columbia, Canada	15 - Lscul	1	DNA	Gary W. Saunders
L. sinclairii	Mud Cove, Bamfield, British Columbia, Canada	16 - GWS001179	1	DNA	Gary W. Saunders
L. yezoensis	Culture: L. Druehl, Bamfield, British Columbia, Canada	17 - Lycul	1	DNA	Gary W. Saunders
Pleurophycus gardneri	Pachena Bay, Bamfield, British Columbia, Canada	23 - CL000903	1	DNA	Gary W. Saunders

Species	Location	Sample code/s	n	Sample type	Collected by
Family Lessoniacea					
Dictyoneurum californicum	Agassiz Beach, California, USA	34- DR01AB	1	DNA	Gary W. Saunders
Lessonia corrugata	Bicheno, Tasmania, Australia	4 - GWS001454	1	DNA	Gary W. Saunders
L. corrugata	North end Blackmans Bay Beach, Tasmania, Australia	Lc1, Lc2, Lc3	3	Frozen	Felicity McEnnulty
L. nigrescens	Las Cruces, Chile	29 - LESS	1	DNA	Gary W. Saunders
L. flavicans	Falklands. from S. Fredericq	30 - SF1597	1	DNA	Gary W. Saunders
Lessoniopsis littoralis	Amphitrite Pt., Ucluet, British Columbia, Canada	18 - LL04AP	1	DNA	Gary W. Saunders
L. littoralis	Kirby PT., Bamfield, British Columbia, Canada	19 - LL01KP	1	DNA	Gary W. Saunders
L. littoralis	Frank I., Tofino, British Columbia, Canada	20 - LL02FI	1	DNA	Gary W. Saunders
L. littoralis	Garapata Beach, Hopkins Marine Center, California, USA.	38-LL06GB	1	DNA	Gary W. Saunders
Macrocystis pyrifera	Tinderbox Beach, Tasmania, Australia	Mp1, Mp2, Mp3	3	Frozen	Felicity McEnnulty
M. angustifolia	Bridport, Tasmania, Australia	Ma1, Ma2, Ma3	3	Frozen	Felicity McEnnulty
M. integrifolia	Spring Cove, Ucluet, British Columbia, Canada	21-MI03SC, 22-MI04SC	2	DNA	Gary W. Saunders
M. integrifolia	Sitka Harbour, Alaska, USA	39-MI02ST, 40-MI05ST	2	DNA	Gary W. Saunders
Nereocystis leutkeana	Garbage Pt. Friday Harbour, Washington, USA	41-NL02GP, 42-NL04GP	2	DNA	Gary W. Saunders
N. leutkeana	Tu Rock, Friday Harbour, Washington, USA	43-NL03TR, 44-NL05TR	2	DNA	Gary W. Saunders
Pelagophycus porra	San Diego, California, USA	45 - PELA	1	DNA	Gary W. Saunders
Postelsia palmaeformis	Botany Beach, Port Renfrew, British Columbia, Canada	24-PP01BA, 25-PP02BA, 26-PP04BA	3	DNA	Gary W. Saunders
P. palmaeformis	Garapata Beach, Hopkins Marine Center, California, USA	46 - PP06GB	1	DNA	Gary W. Saunders

APPENDIX 2 RIBOSUMAL LSU REGION

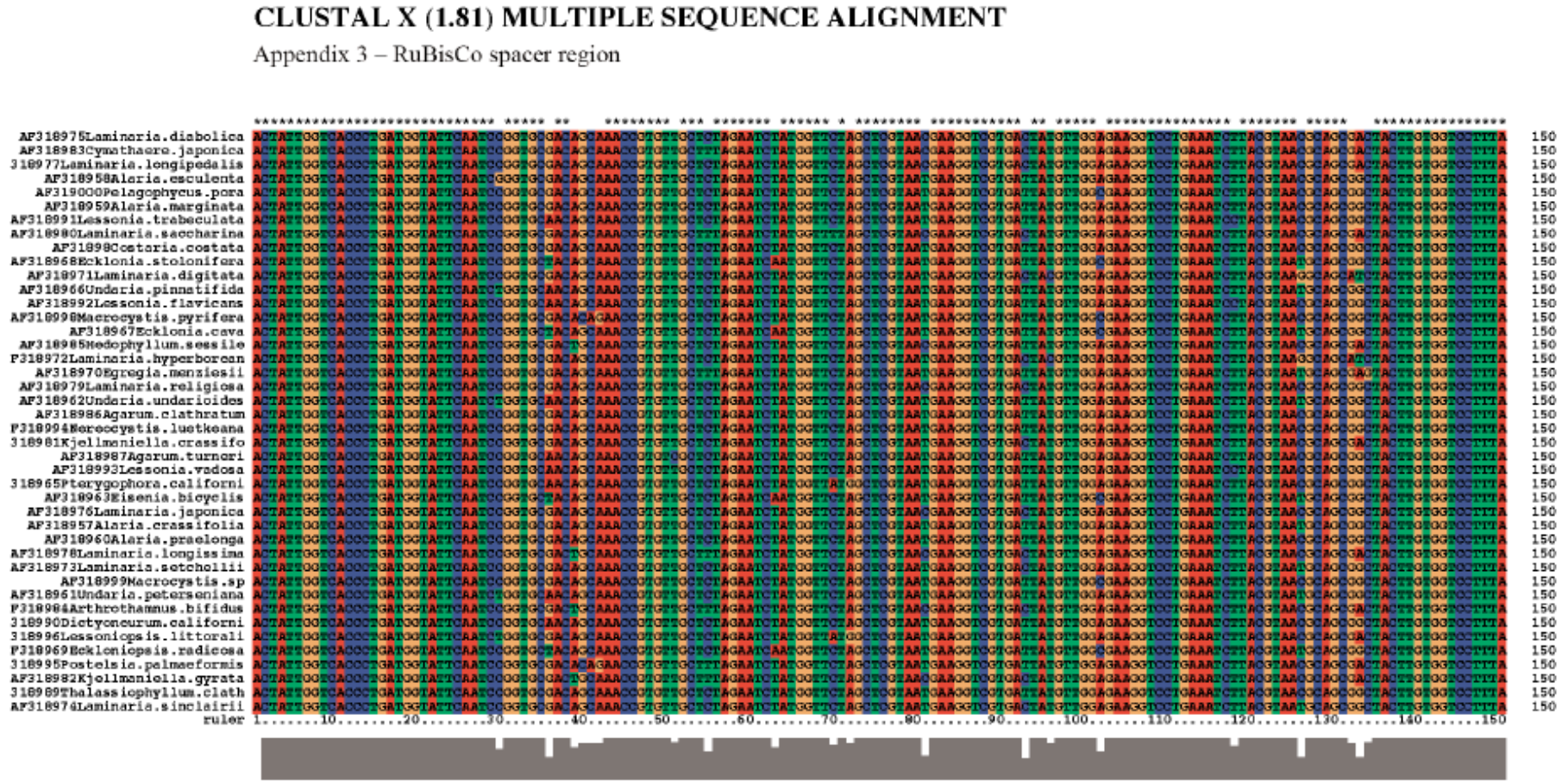


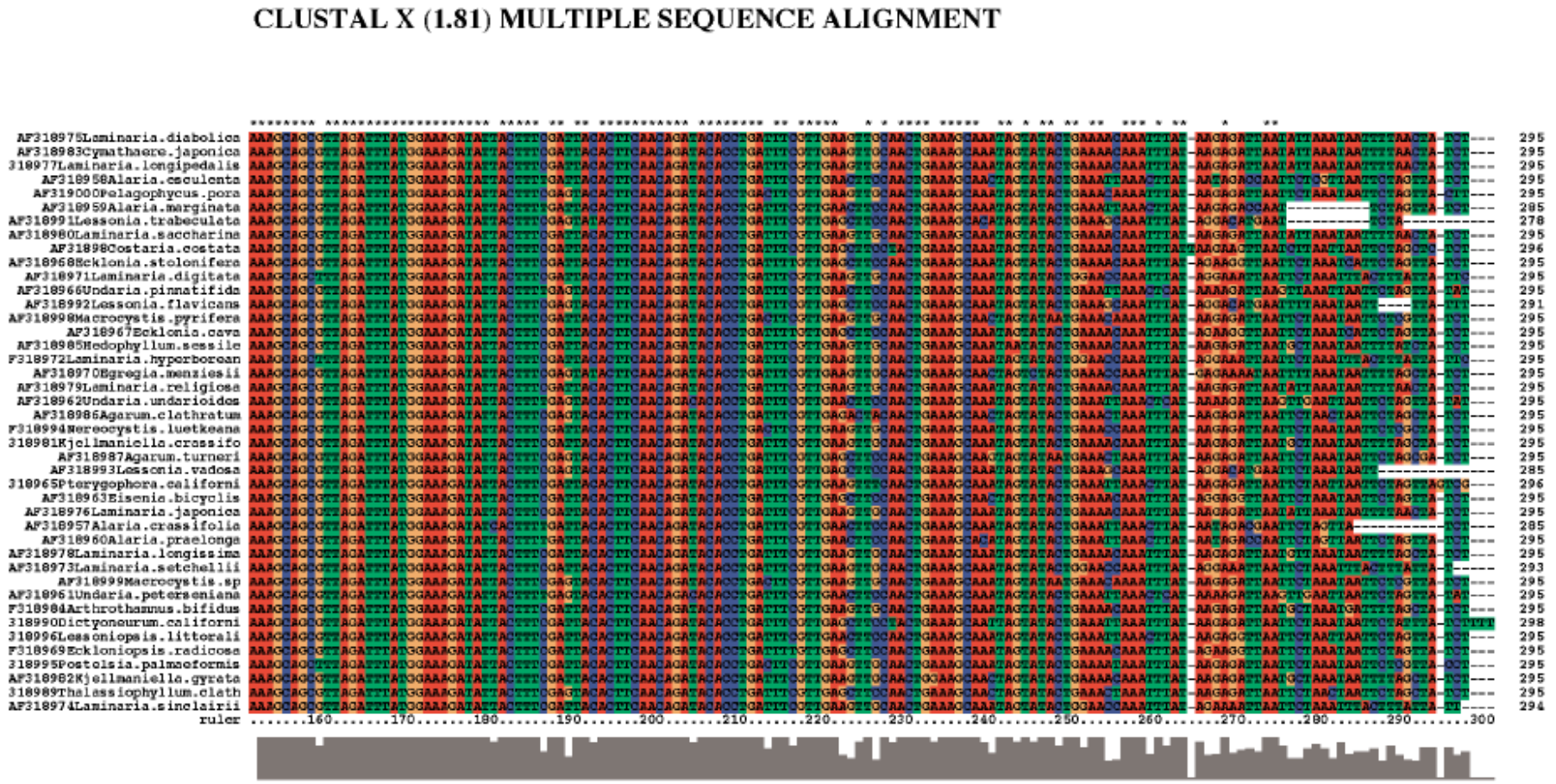
Empirical validation: Stage II



Empirical validation: Stage II

APPENDIX 3 RUBISCO SPACER REGION





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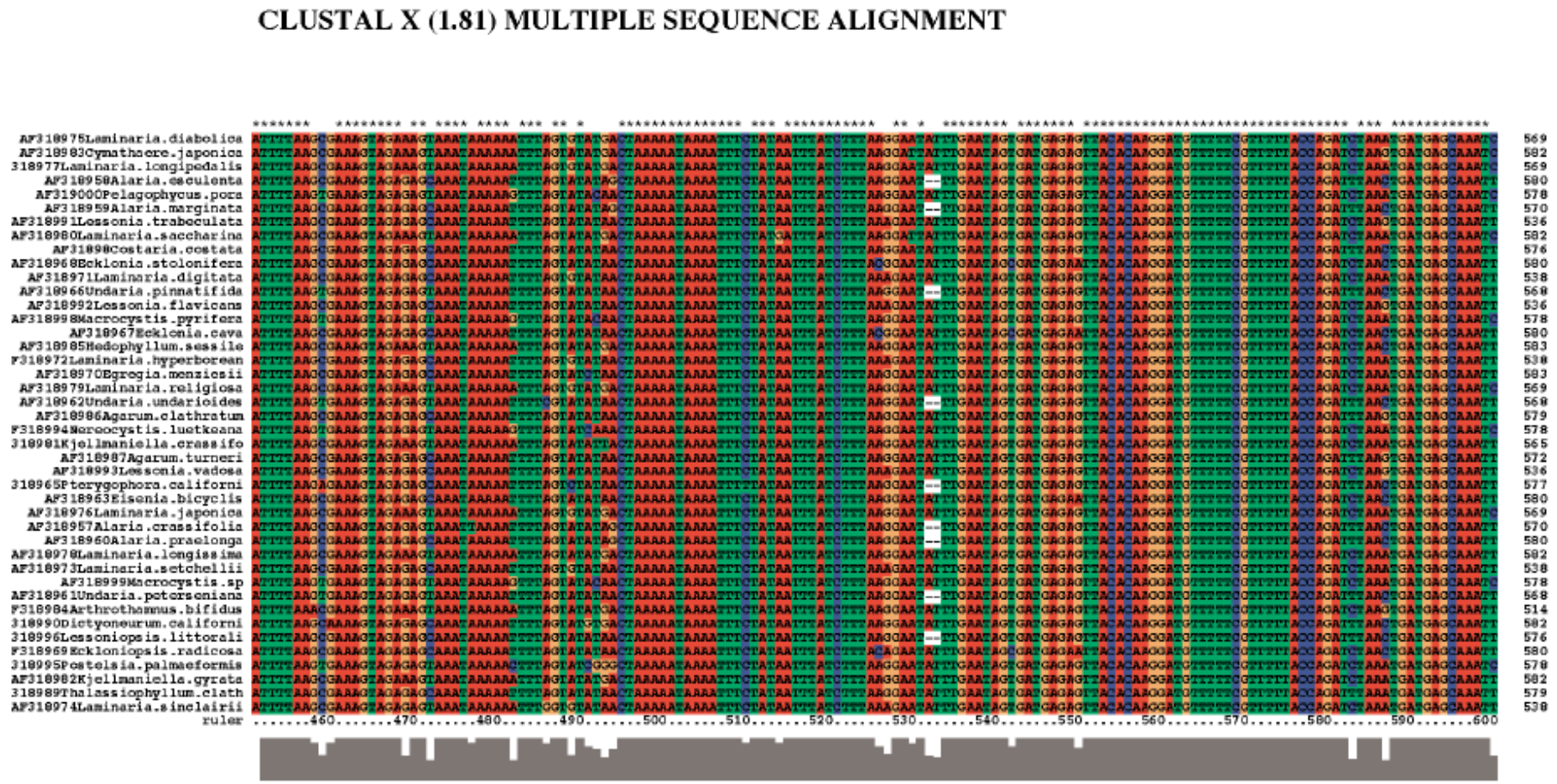

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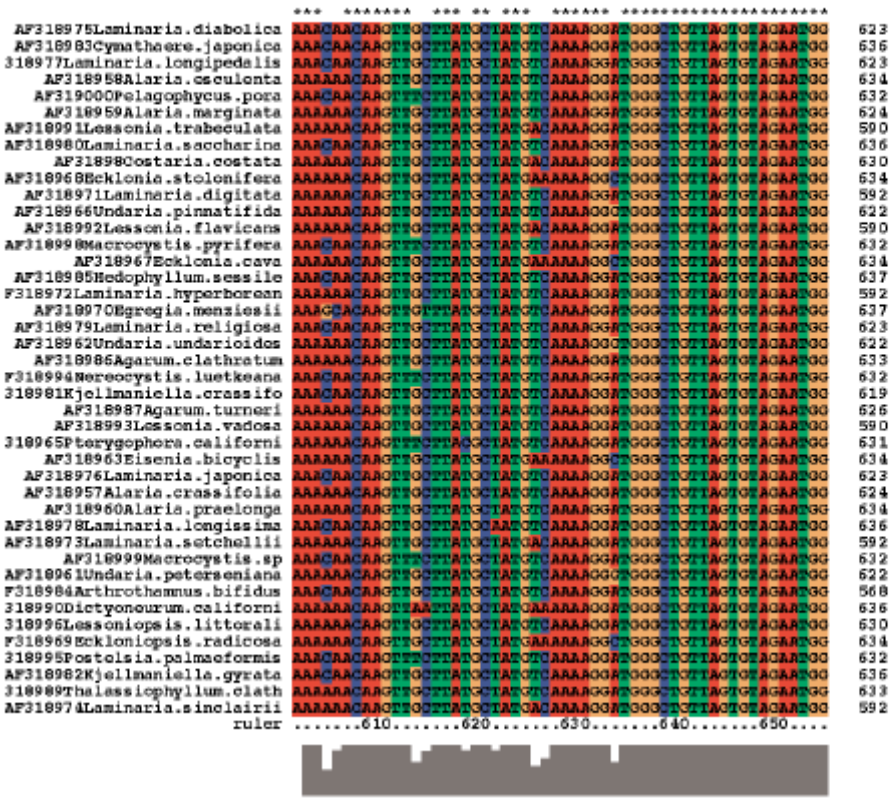
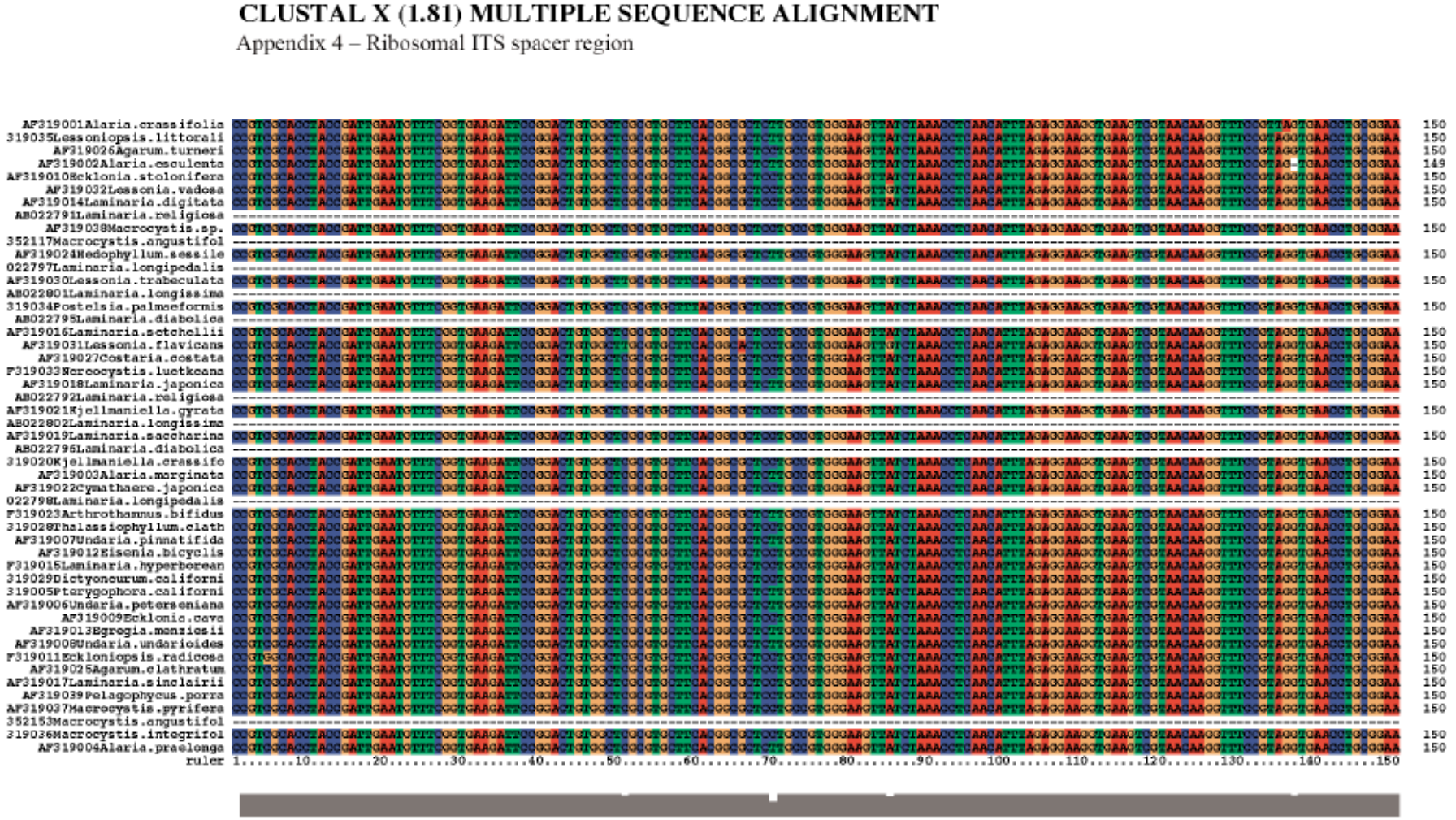
CLUSTAL X (1.81) MULTIPLE SEQUENCE ALIGNMENT



APPENDIX 4 RIBOSOMAL ITS SPACER REGION



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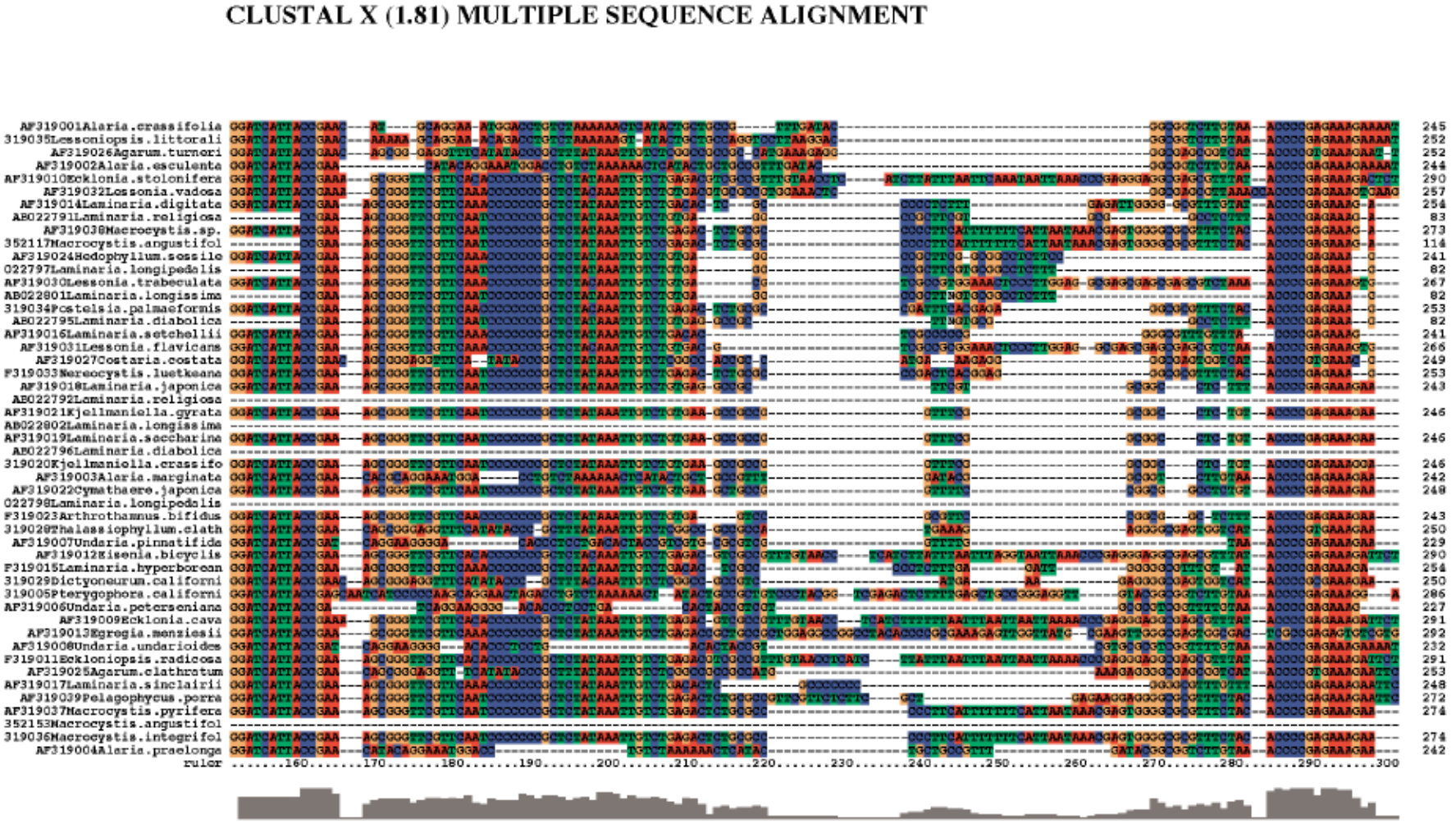

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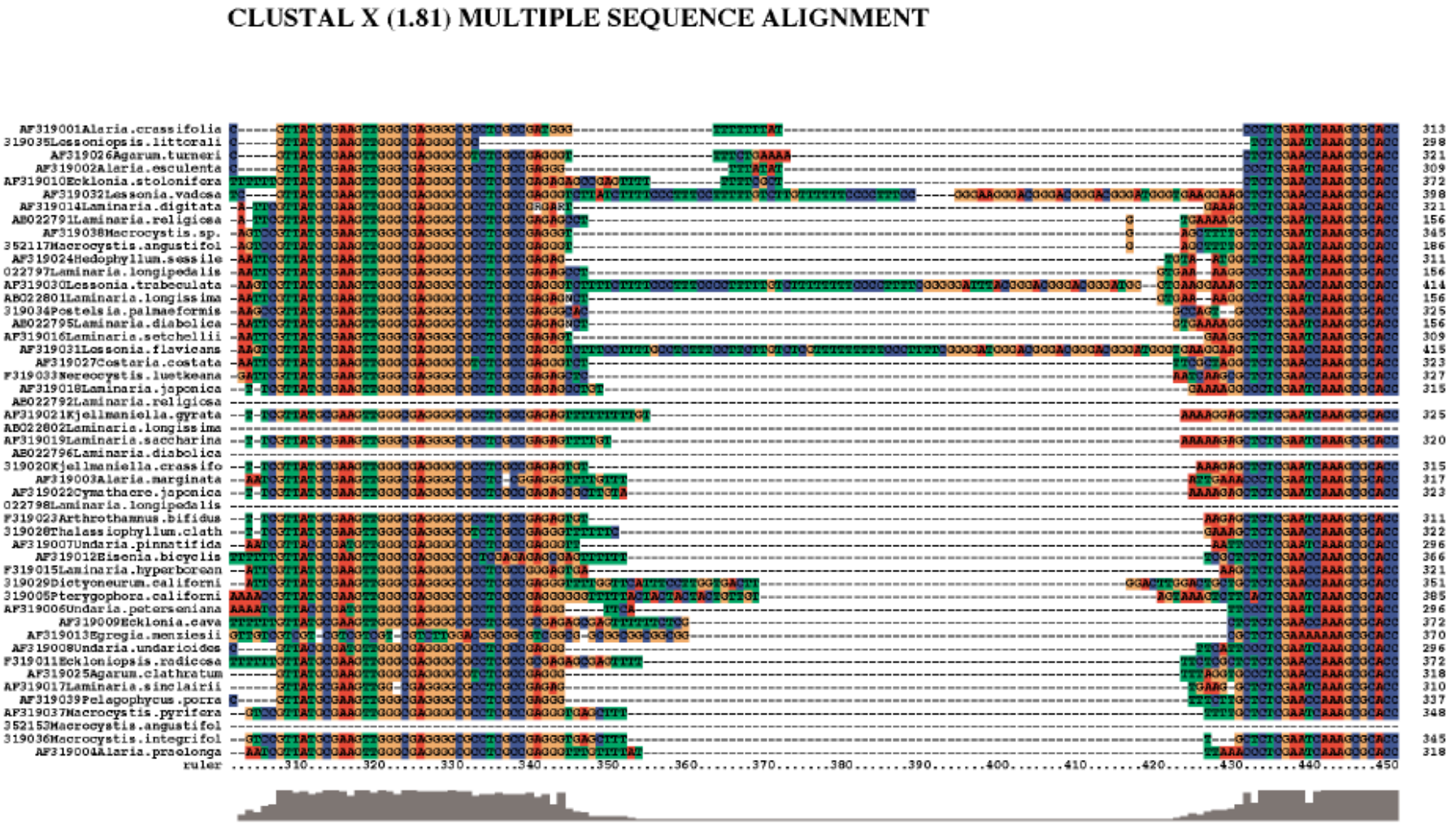

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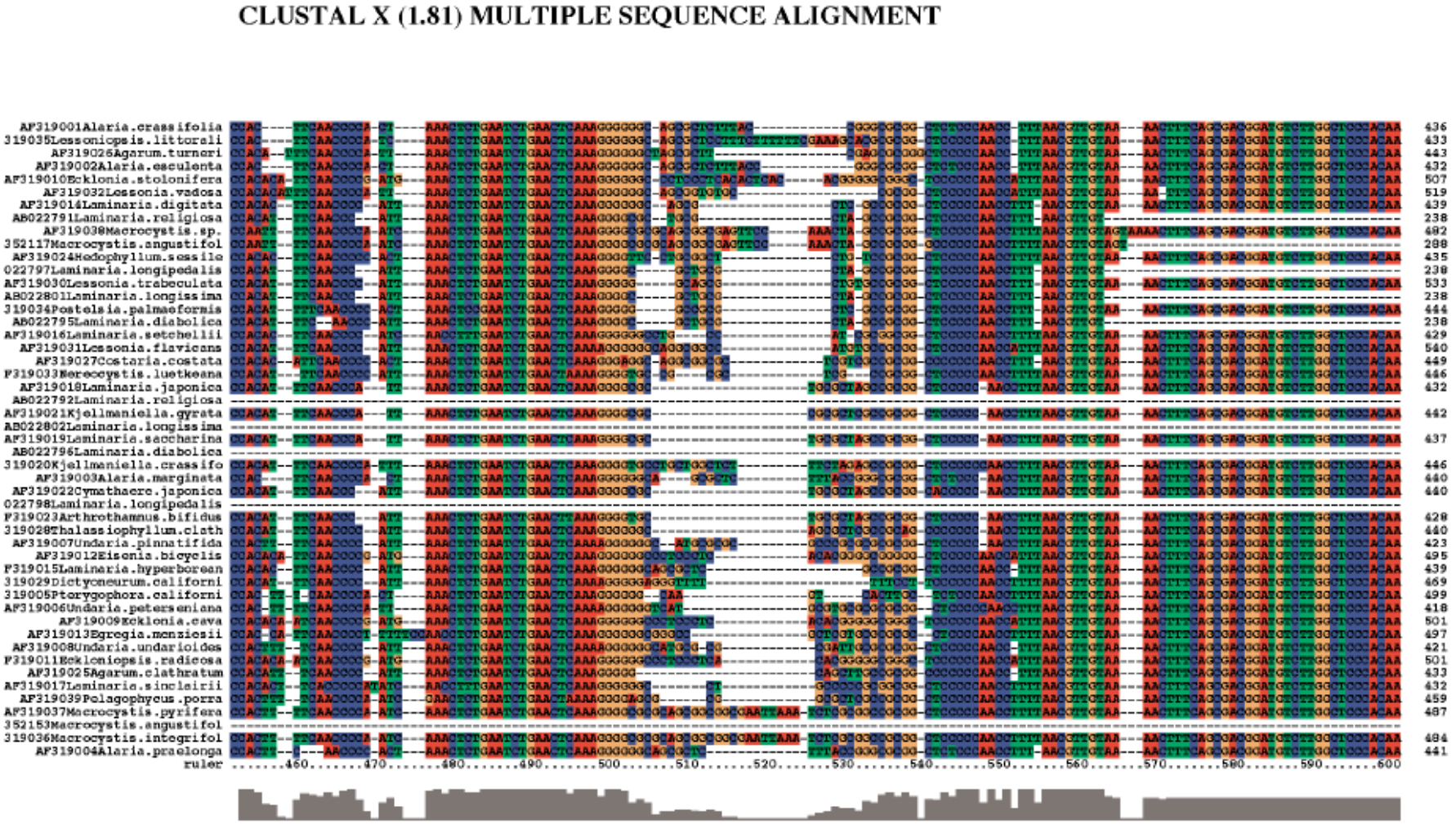

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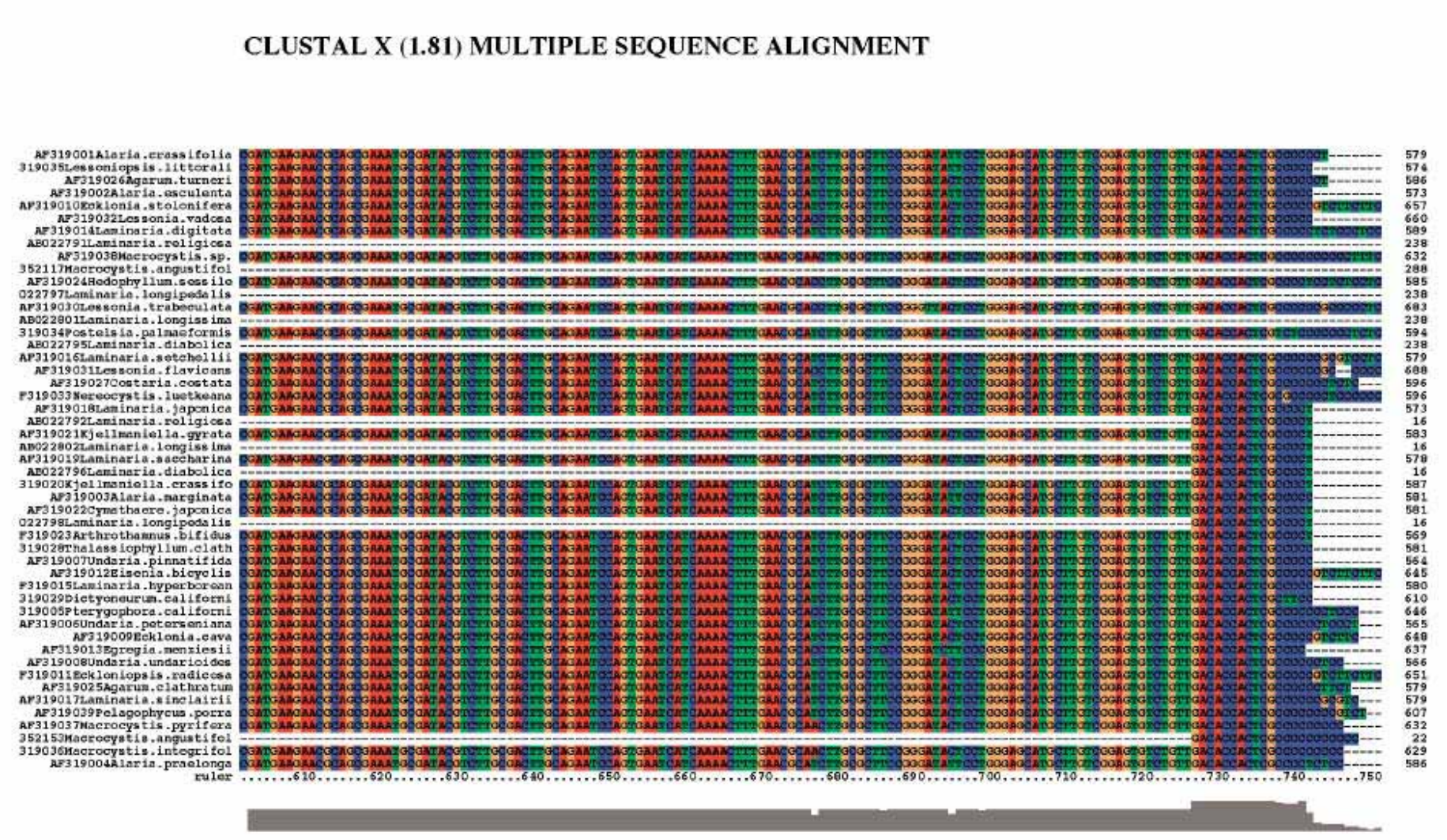

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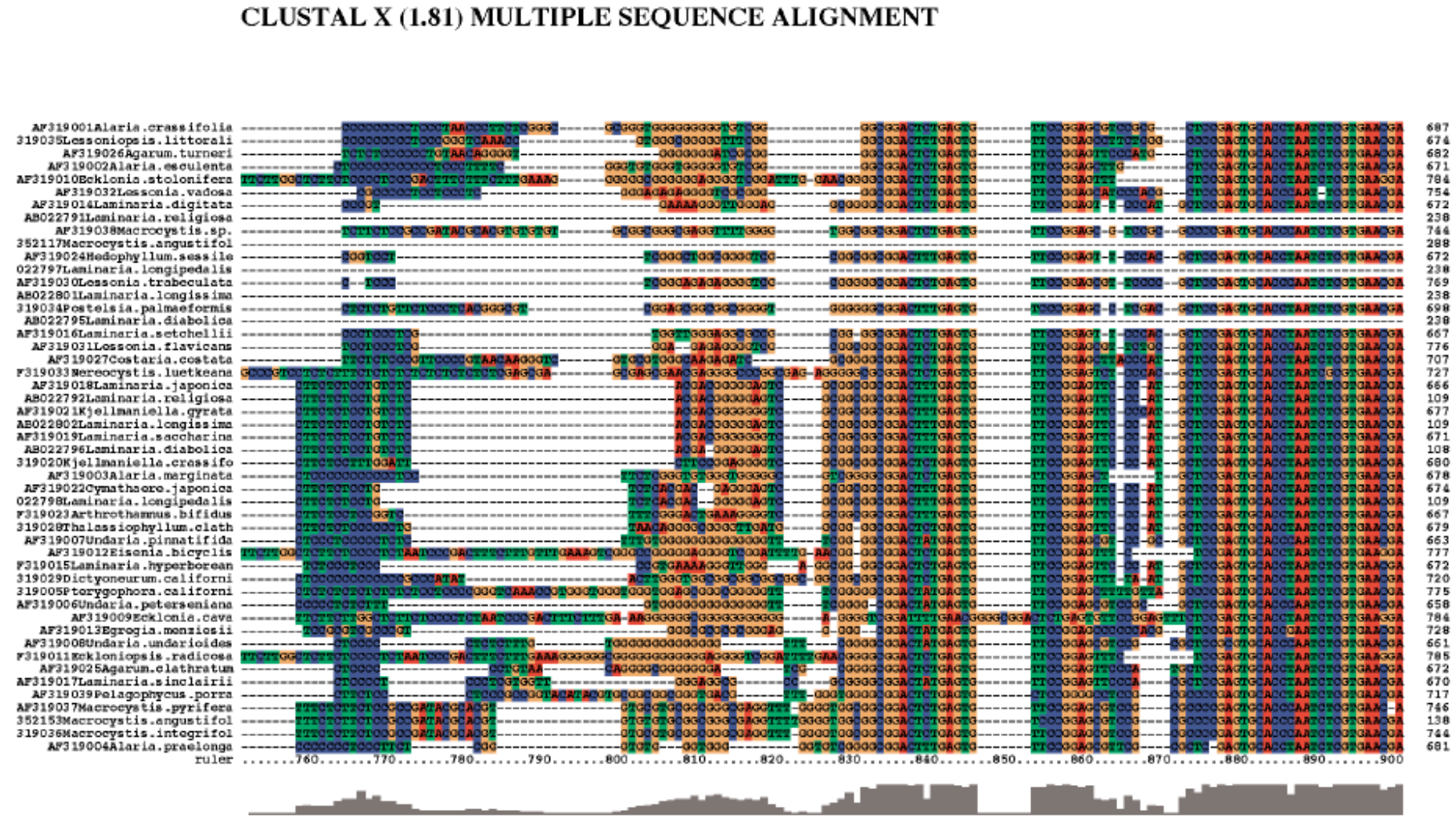

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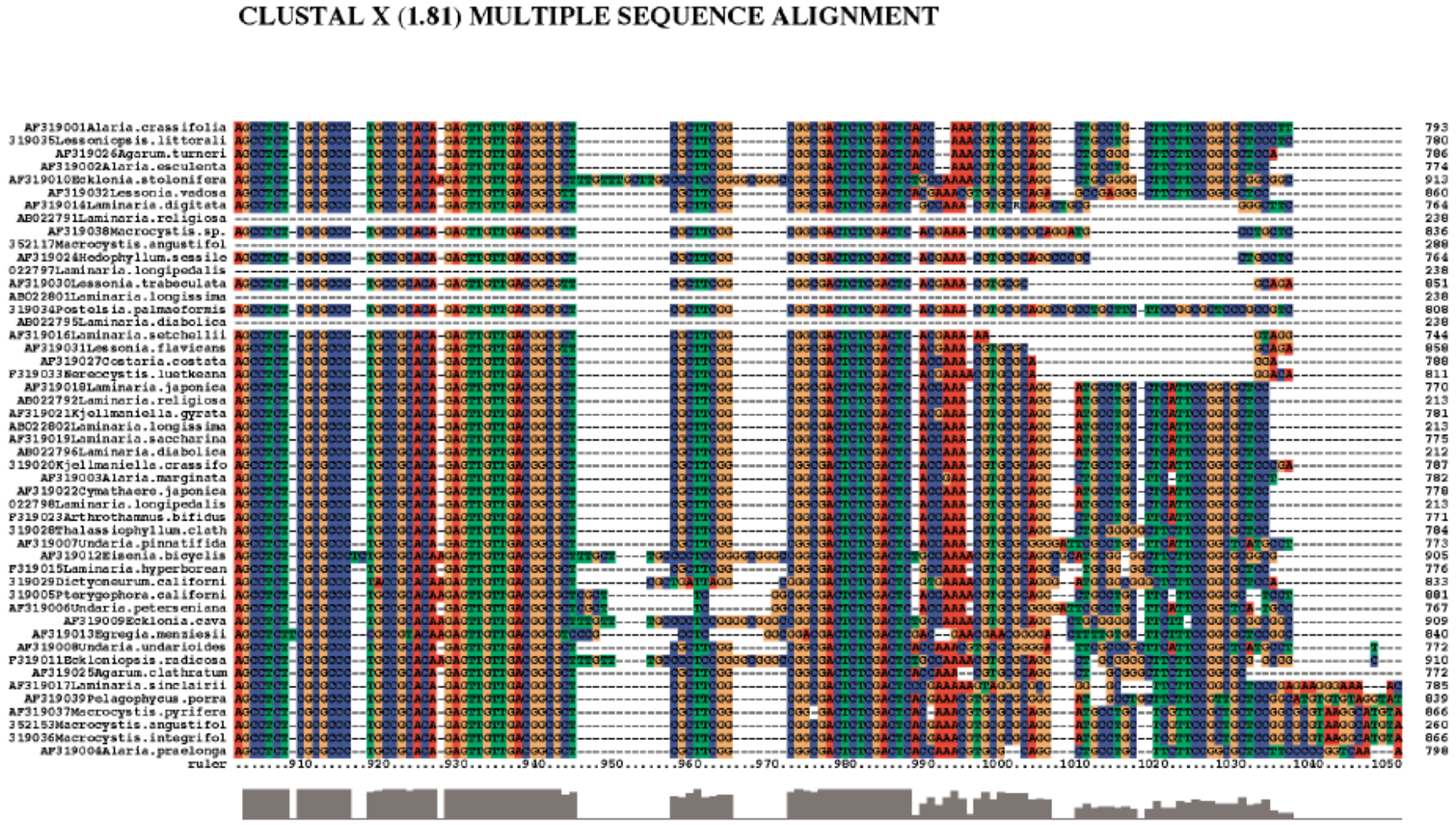

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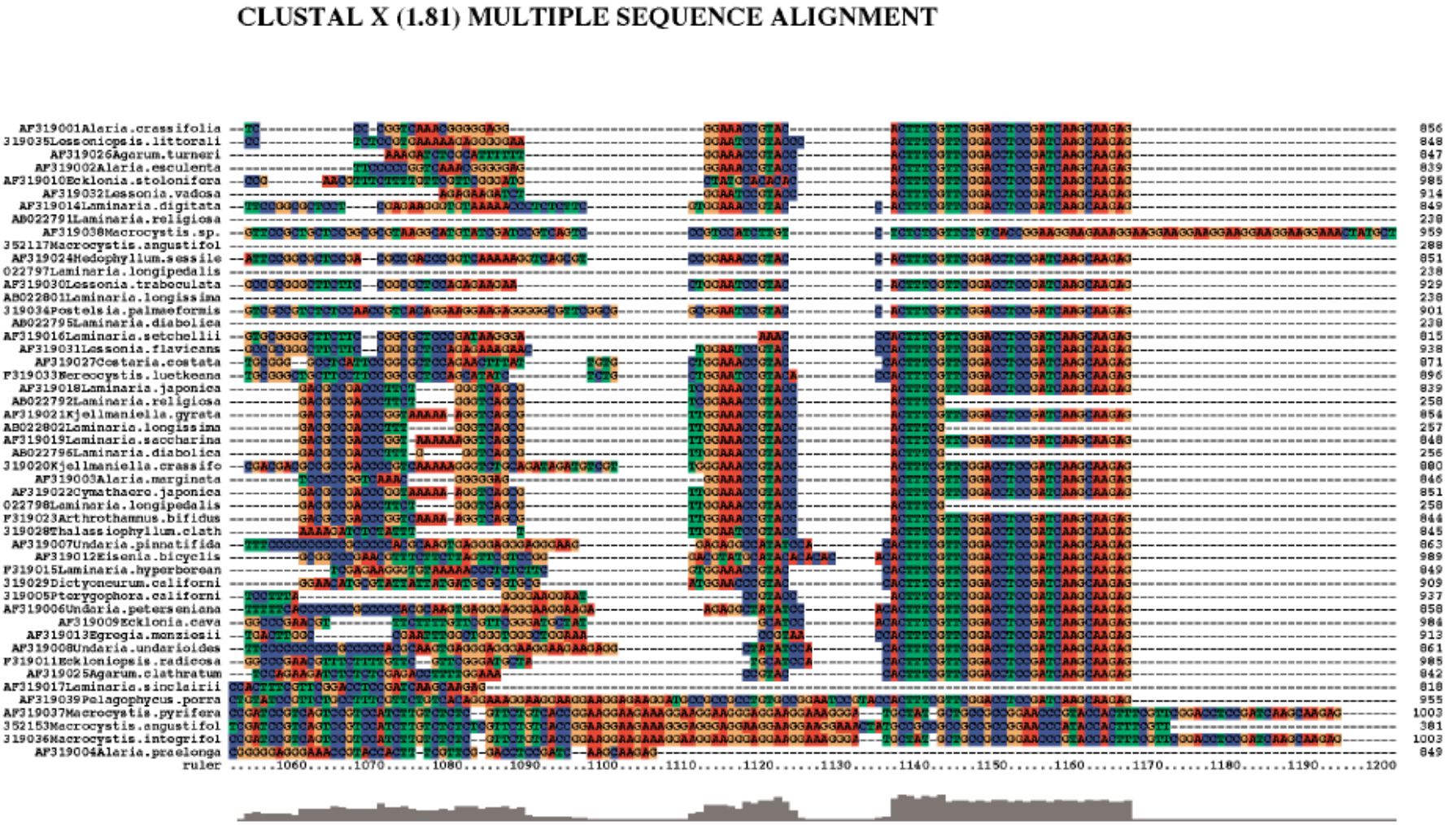


Empirical validation: Stage II

APPENDIX 5 PROJECT SUMMARY SHEET

Small Vessel Translocation of Key Threatening Species

Background Information

Humans are transporting organisms and introducing them to new areas at an unprecedented rate. Introductions of non-native species are a serious threat to the diversity of plants and animals as they invade natural areas and replace native species. Hull fouling of yachts and fishing boats is contributing to the introduction and spread of non-native species to new sites. The 1999 black-striped mussel incursion in Darwin is a recent example. It was first introduced on the hull of an ocean-going yacht, and then spread to nearby marinas by other local yachts.

In southeast Australia, introduced marine pests such as the northern Pacific sea star, *Asterias amurensis* and Japanese kelp, *Undaria pinnatifida* are a serious threat. The northern pacific sea star is an important predator of scallops and mussels, while Japanese kelp fouls aquaculture facilities increasing operation costs and may hinder abalone and urchin divers. These "key threatening species" are spreading around the coastlines of southeast Australia. Recreational vessels may be inadvertently assisting this process.

What Are We Doing?

The CSIRO is undertaking a study to assess the spread of these two introduced pests via small vessels from their main population centres in Tasmania and Victoria to other uninfected regions. Both *Undaria* and *Asterias* have a water borne and settled stage, therefore we are interested in collecting fouling and water samples. We will be taking samples from wet internal surfaces such as bilges and chain lockers, and from external surfaces such as the hull and keel, which will be scrapped using both rubber and metal scrapers while the boat is slipped.

We will be targeting recreational vessels, fishing vessels and aquaculture facilities that operate in areas where the northern Pacific sea star and/or Japanese kelp occur in high densities, such as the Derwent estuary.

For More Information…

If you would like to know more about CSIRO Marine Research and introduced species, visit http://crimp.marine.csiro.au/ or contact Keith Hayes on 6232 5260 or Caroline Sutton on 6232 5386 to discuss the current project.

APPENDIX 6 SURVEY SHEETS

A. VESSEL & OWNER DETAILS

Date:	Location:
Vessel name:	Vessel type:
Vessel registration:	Owner/skipper name:
Owner/skipper tel:	Vessel length (m/ft):
Vessel draft (m/ft):	Vessel beam (m/ft):
Displacement (tonnes):	Length on water line (m/ft):
Hull material(s):	Length between perpendiculars (m/ft):

B. ACTIVITY DETAILS

Location(s) vessel is usually moored:	
Max. # of trips per annum:	Min. # trips per annum:
Max. trip duration (days):	Min. trip duration (days):
Max. trip distance (kms):	Min. trip distance (kms):
Max. # of stops per trip:	Min. # of stops per trip:
Date of last trip:	Destination:
Antifouling name:	Date last antifouled:
Date last slipped:	Date last in water hull cleaning:

Boat name: **Date:** **Samplers initials:**

C. HULL FOULING RATING

	HULL SECTION		
	Boot-top	Vertical bottom	Flat bottom
PORT			
STARBOARD			

0 A clean, foul-free surface

10 Continuous and gradual gradations of shades of red and green (incipient slime)

20 Slime as dark green patches with yellow and/or brown coloured areas (advanced slime)

30 Weed as filaments up to 3 in. in length, projections up to 1/4 in. in height; or a flat network of filaments, green, yellow, or brown in colour

40 Calcareous fouling on edges, welded seams, corners, or as discrete patches covering flat areas roughly 9 to 10 in. in diameter

50 Random and scattered distribution of fouling by marine animals** on slightly curved or flat surfaces

60 Area distribution of fouling by marine animals 1/4 in. in diameter or less; fouling does not completely cover or blank out surface

70 Fouling by marine animals 1/4 in diameter or less that completely covers surface in patches exceeding 9 to 10 in. in diameter; with radiating fringes of fouling growth

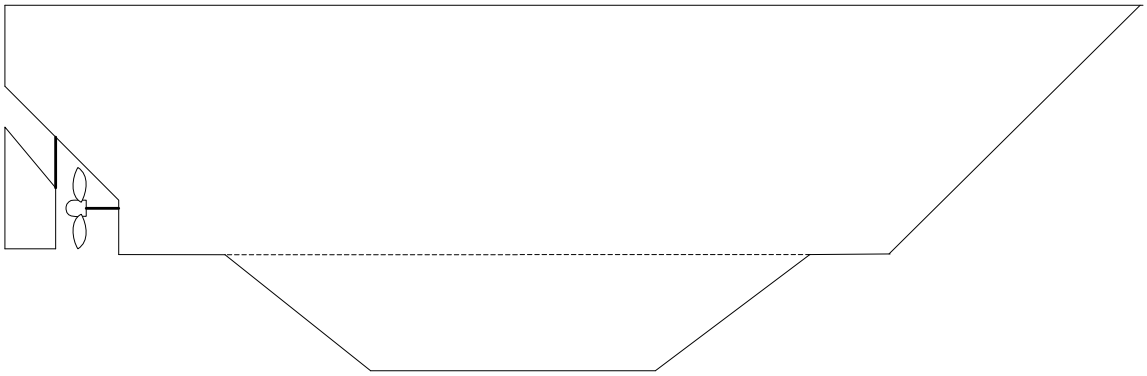
80 Dense fouling by marine animals with upright growth away from hull surface, calcareous shells appear clean or white in colour

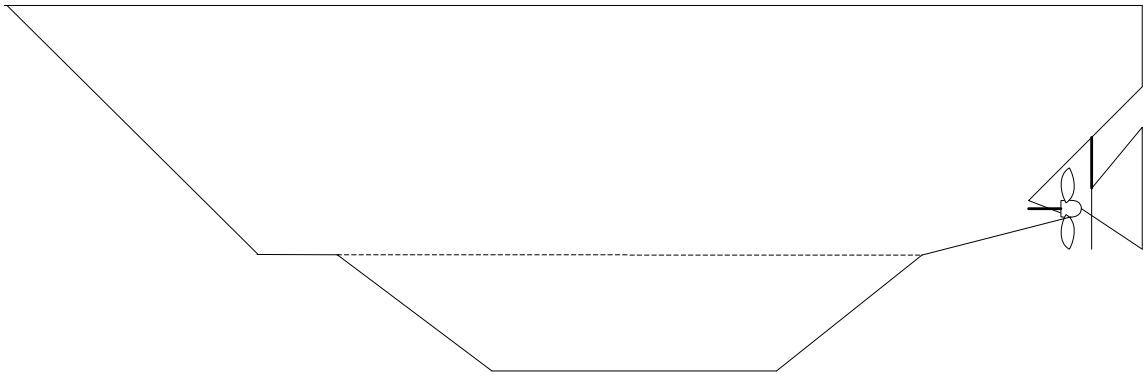
90 Dense fouling by marine animals with upright growth away from hull surface, calcareous shells brown in colour or with slime and/or weed overlay

100 All forms of fouling present particularly soft sedentary animals without calcareous covering

** Fouling marine animals typically include tubeworms, barnacles, bryozoans, hydroids, sea squirts and molluscs.

Boat name: **Date:** **Samplers initials:**





Boat name: **Date:** **Samplers initials:**

Vessel survey record – Hull

CODE	LOCATION	SAMPLE	PHOTO	NOTES
HA	Hull Surface: External			
HB	Sonar tubes: Internal			
HC	Skin fittings: External			
HD	Water inlet/outlet cover plates: External			
HE	Depth sounder booth: Internal			
HF	Keel cooling pipes: External			
HG	Water inlet/outlet cover plates: Internal			
HH	Depth sounder booth: External			
HI	Bob-stay fitting: Internal			
HJ	Paddle wheel and booth: Internal			
HK	Paddle wheel and booth: External			
HL	Block space: External			
HM	Transducer: External			
HN	Keel - retractable: External			
HO	Keel - retractable: Internal			
HP	Keel - fixed: External			
HQ	False keel: External			
HR	Stabilisers/trim tabs - folding: Top			
HS	Stabilisers/trim tabs - folding: Bottom			
HT	Rolling chock - fixed: Top			
HU	Rolling chock - fixed: Bottom			
HV	Head fitting: External			
HW	Head fitting: Internal			
HX	Garboard plank: External			
HY	Bob-stay fitting: External			
HZ	Marlin board: External			
HAA	Zinc blocks: Behind			
HAB	Zinc blocks: Front			
HAC	Exhaust outlet: External			
HAD	Exhaust outlet: Internal			
HAE	Autopilot sensor			
HAF	Radio earth plate: External			
HAG	Live catch tank – inlet/outlet: Internal			
HAH	Boot-top (1m scrape)			
HAI	Transom (1m scrape)			

Boat name: **Date:** **Samplers initials:**

Vessel Survey Record – Propeller, rudder & anchor

CODE	LOCATION	SAMPLE	PHOTO	NOTES
PA	Propeller Shaft: External			
PB	Propeller Surface: External			
PC	Propeller nozzle: External			
PD	Outboard sail drive legs: Internal			
PE	Outboard sail drive legs: External			
PF	Stern tubes cover/stern gland: Internal			
PG	Stern tubes cover/stern gland: External			
PH	Keel extension/Skeg: External			
PI	Keel extension/Skeg: Internal			
PJ	Rudder surface: External			
PK	Tiller flat: External			
PL	Tiller flat: Internal			
PM	Anchor Surface: External			
PN	Chain: External			
PO	Sea anchors/parachutes: External			
PP	Sea anchors/parachutes: Water			
PQ	Anchor buoys: External			
PR	Anchor buoys: Water			
PS	Propeller cowling: external			
PT	Rudder pintel: External			
PU	Rudder cavity			
PV	Engine mount: External			

Boat name: **Date:** **Samplers initials:**

Vessel Survey Record – Internal spaces

CODE	LOCATION	SAMPLE	PHOTO	NOTES
IA	Seawater/grey-water inlet/outlets: Water			
IB	Seawater/grey-water inlet/outlets: Internal			
IC	Sewage holding tank: Water			
ID	Bilge - closed: Water			
IE	Bilge - open: Water			
IF	Engine cooling water filter: Water			
IG	Engine cooling water filter: Internal			
IH	Anchor well: Water			
II	Anchor well: Sediment			
IJ	Ballast tanks/brine storage tanks: Water			
IK	Ballast tanks/brine tanks: Sediment			
IL	Live catch wet well: Water			
IM	Live catch wet well: Internal			
IN	Live catch circulation tank: Internal			
IO	Live catch circulation tank: Water			
IP	Rudder control room: Water			
IQ	Rudder control room: Internal			
IR	Ice makers (sea water): Water			
IS	Shower holding tank: Water			
IT	Storage rooms: Water			
IU	Storage boxes: Water			
IV	Cockpit/Wheelhouse: Water			
IW	Cockpit/Wheelhouse: Sediment			
IX	Dead catch storage – spray room: Water			
IY	Dead catch storage – insulated: Water			

Boat name: **Date:** **Samplers initials:**

Vessel Survey Record – Fishing gear

CODE	LOCATION	SAMPLE	PHOTO	NOTES
FA	Net - beach seine: Water			
FB	Net - beach seine: External			
FC	Net - purse: Water			
FD	Net - purse: External			
FE	Net - gill: Water			
FF	Net - gill: External			
FG	Net - trawl: Water			
FH	Net - trawl: External			
FI	Net - dip: Water			
FJ	Net - dip: External			
FK	Net reels: Water			
FL	Net reels: External			
FM	Trawl boards: External			
FN	Floats - pots: Water			
FO	Floats - pots: External			
FP	Trap ropes: External			
FQ	Traps - octopus: Water			
FR	Traps - octopus: External			
FS	Traps - octopus: Internal			
FT	Traps - Cray/king crab: Water			
FU	Traps - Cray/king crab: External			
FV	Traps - Cray/king crab: Internal			
FW	Traps - crab: Water			
FX	Traps - crab: External			
FY	Traps - crab: Internal			
FZ	Dingy/seine tender boat: Water			
FAA	Dingy/seine tender boat: External			
FAB	Dingy/seine tender boat: Internal			
FAC	Marker buoys: Water			
FAD	Marker buoys: External			
FAE	Floats - nets: Water			
FAF	Floats - nets: External			
FAG	Scallop harvesters: External			
FAH	Long lines: External			
FAI	Jigging machines (squid): External			
FAJ	Hooker hoses: External			
FAK	Dive gear: Water			
FAL	Dive gear: External			

Boat name: **Date:** **Samplers initials:**

Vessel Survey Record – Deck

CODE	LOCATION	SAMPLE	PHOTO	NOTES
DA	Cracks in deck/between plates: Water			
DB	Hawser pipe: Sediment			
DC	Gunwale (toe rail): Sediment			
DD	Hatches: Water			
DE	Cockpit bins/open storage: Water			
DF	Winch box: Water			
DG	Surface: Water			
DH	Canvas screens: Water			
DI	Bullwarks: Sediment			
DJ	Net chute: Sediment			
DK	Cutting boards: External			

Plankton Sample Record

CODE	LOCATION	SAMPLE DURATION	NOTES (PUMP OR TOW)
1			
2			
3			

APPENDIX 7 HULL SURFACE AREA CALCULATIONS

The wetted surface area (S) of 'fast' (e.g. racing yachts) hulls is given by Taylor's formula:

$$S = C\sqrt{D_T L_I} \quad , \qquad [A1]$$

where C is a hull shape constant which varies between 2.56 and 2.59, D_T is the vessel displacement (tonnes) and L_I is the mean immersed length (in metres) usually interpreted as the waterline length. For 'slower' hulls with a fuller shape (e.g. recreational yachts and fishing vessels), the wetter surface area is given by Denny's formula:

$$S = 1.7 L_P T + \frac{D_V}{T} \qquad [A2]$$

where L_P is the length between perpendiculars (metres), T is the moulded mean draught (metres) and D_V is the volume of displacement (cubic metres) (http://www.dynagen.co.za/eugene/hulls/).

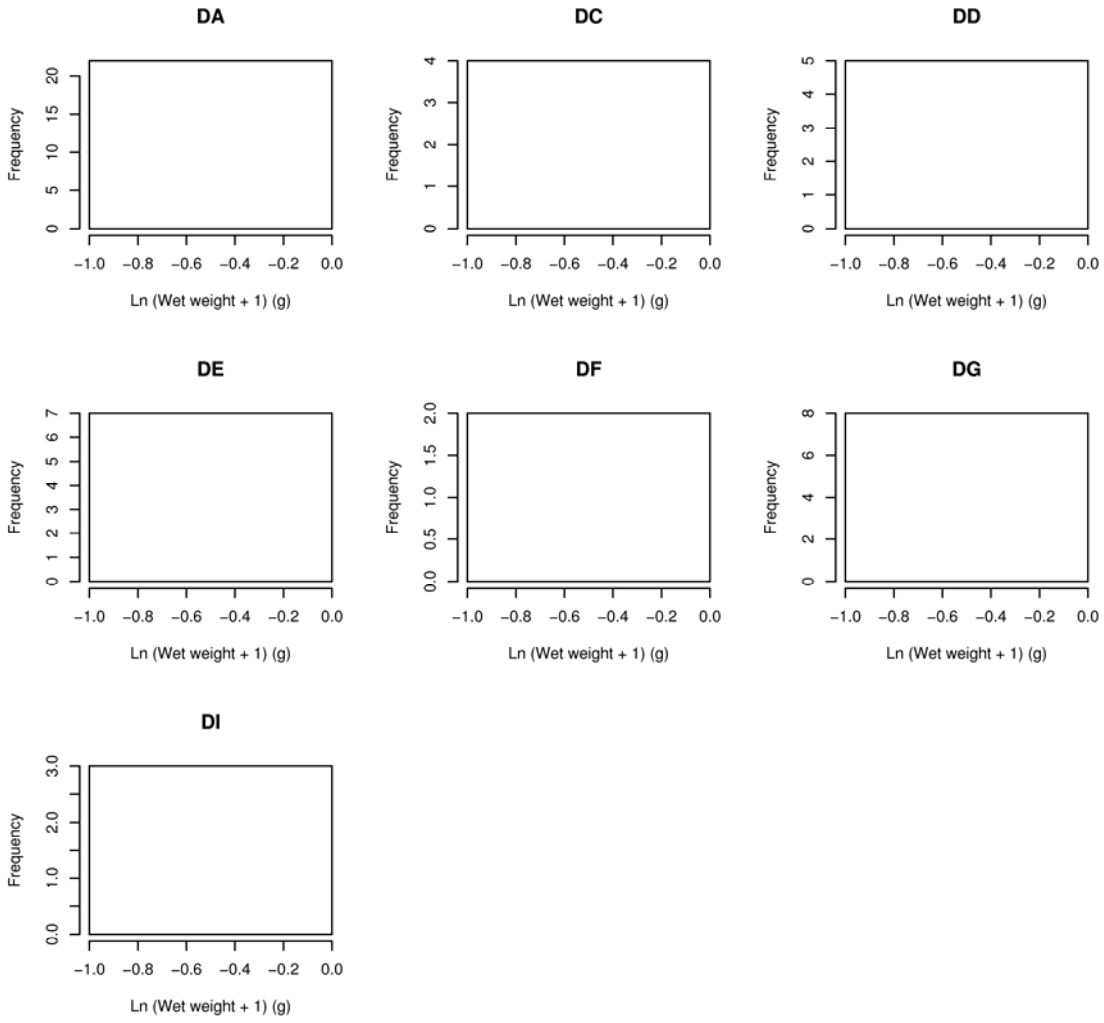
In practise vessel owners, skippers and contractors rarely know the length between perpendiculars or the mean immersed length of their vessels. The wetted surface area of a hull is therefore calculated using the following approximate formula:

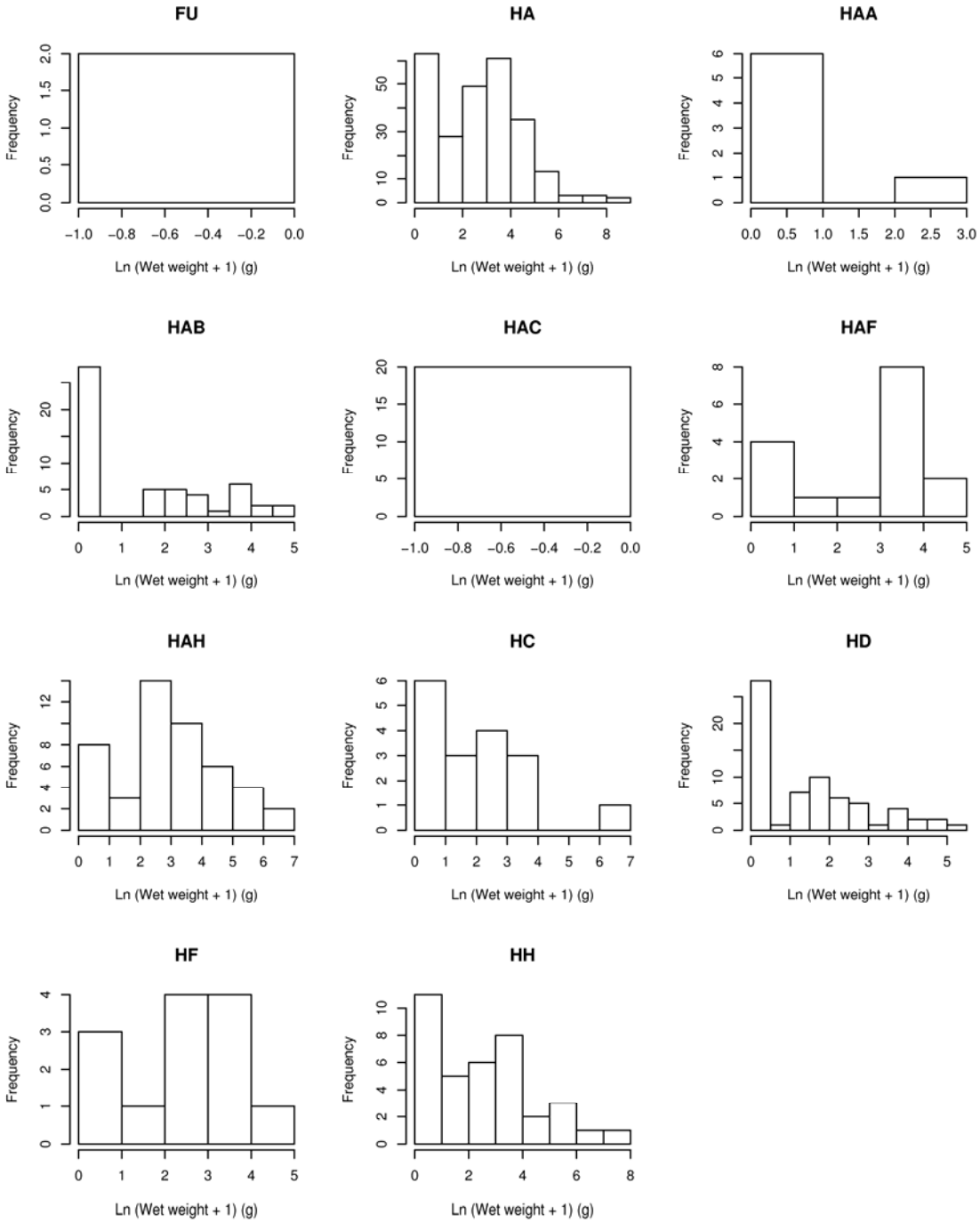
$$S = 2LT \qquad \{A3]$$

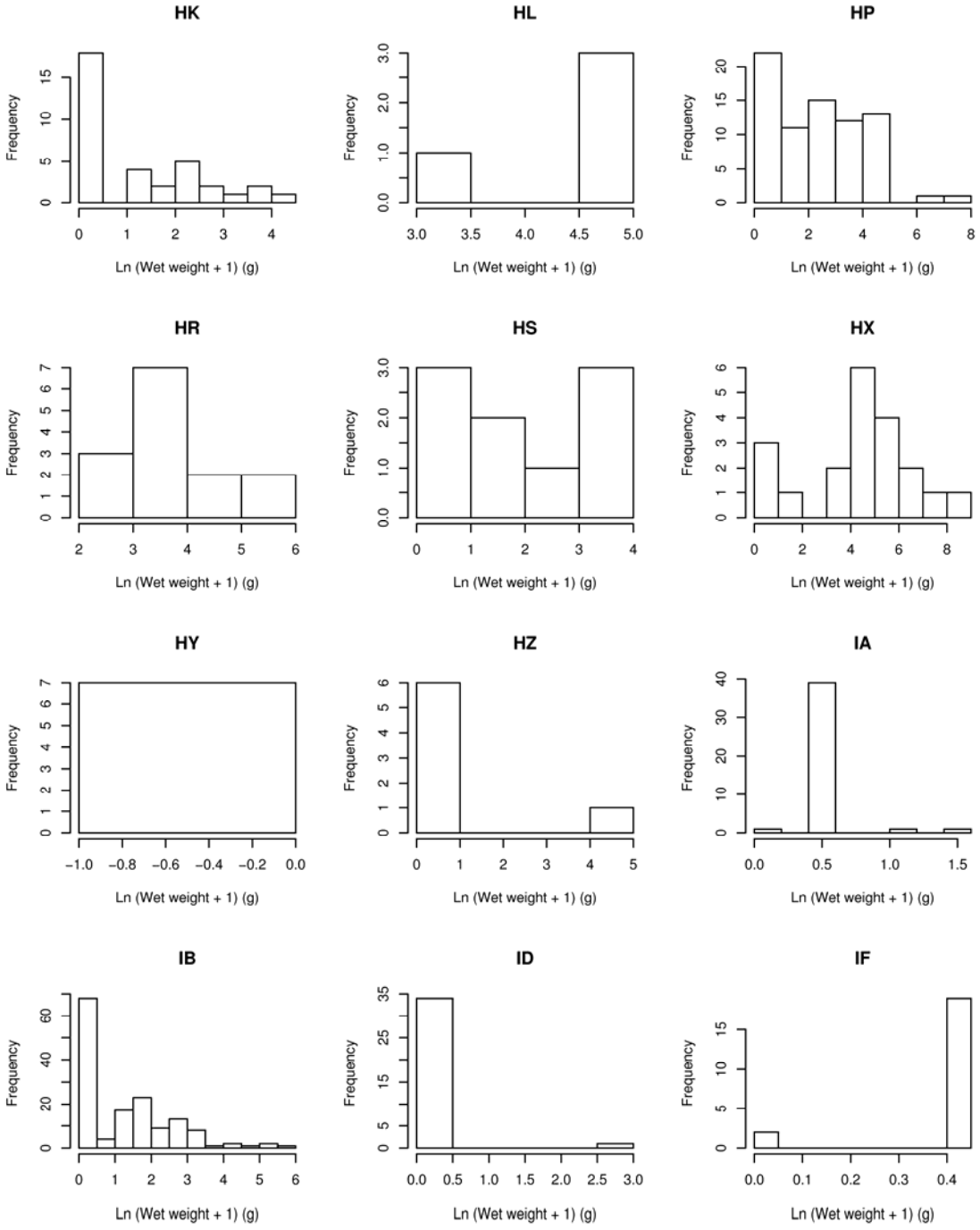
where L is the vessel length (metres) and T is the mean draught (metres) (*pers comm.* C. Hewitt, MAFF).

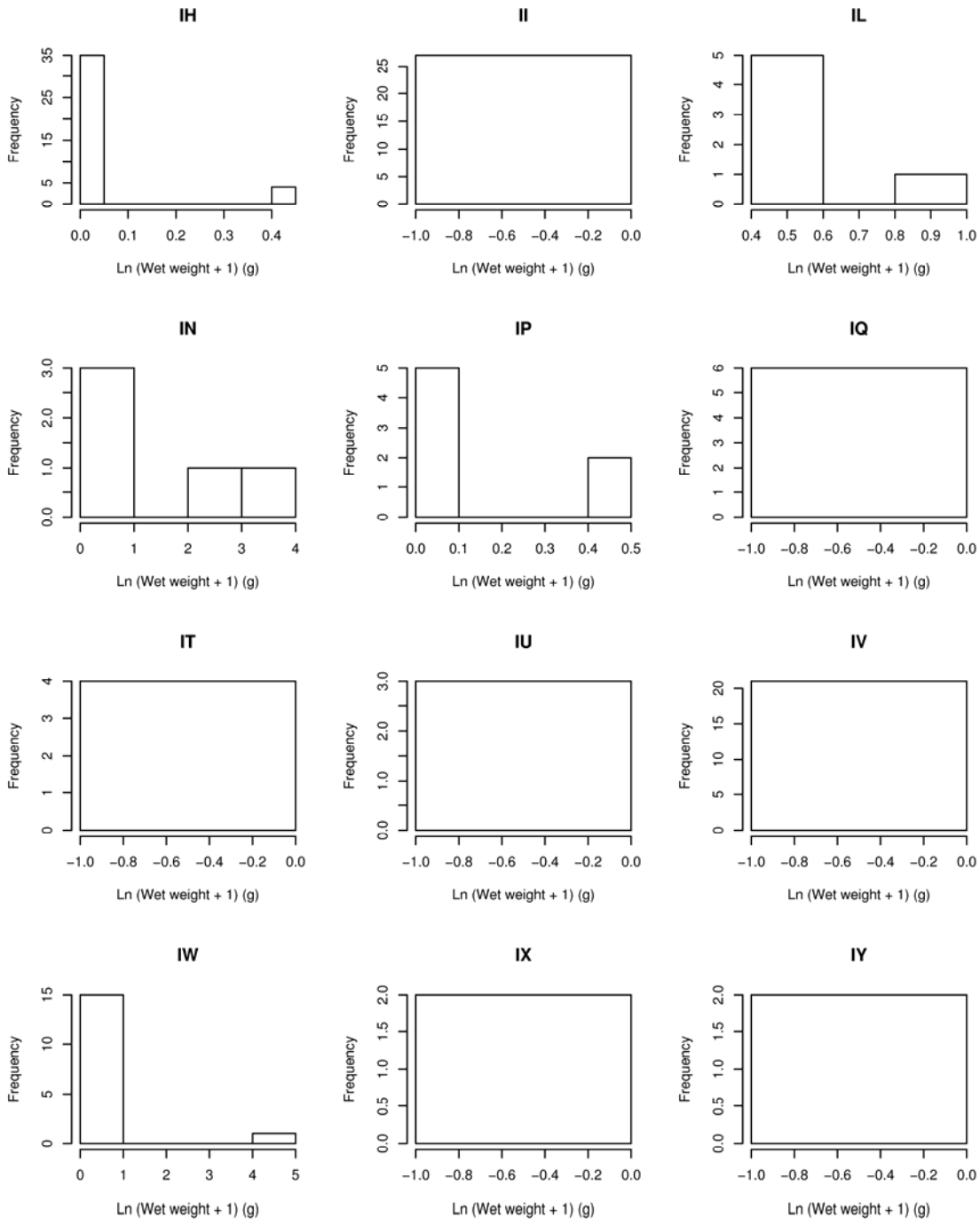
APPENDIX 8 FOULING PATTERNS BY LOCATION

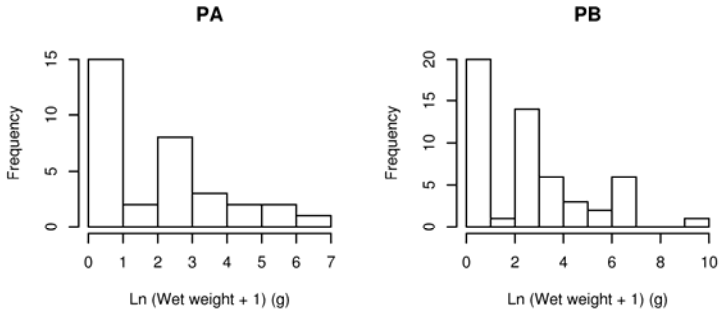
Histograms of the log transformed wet weight of fouling biomass by location

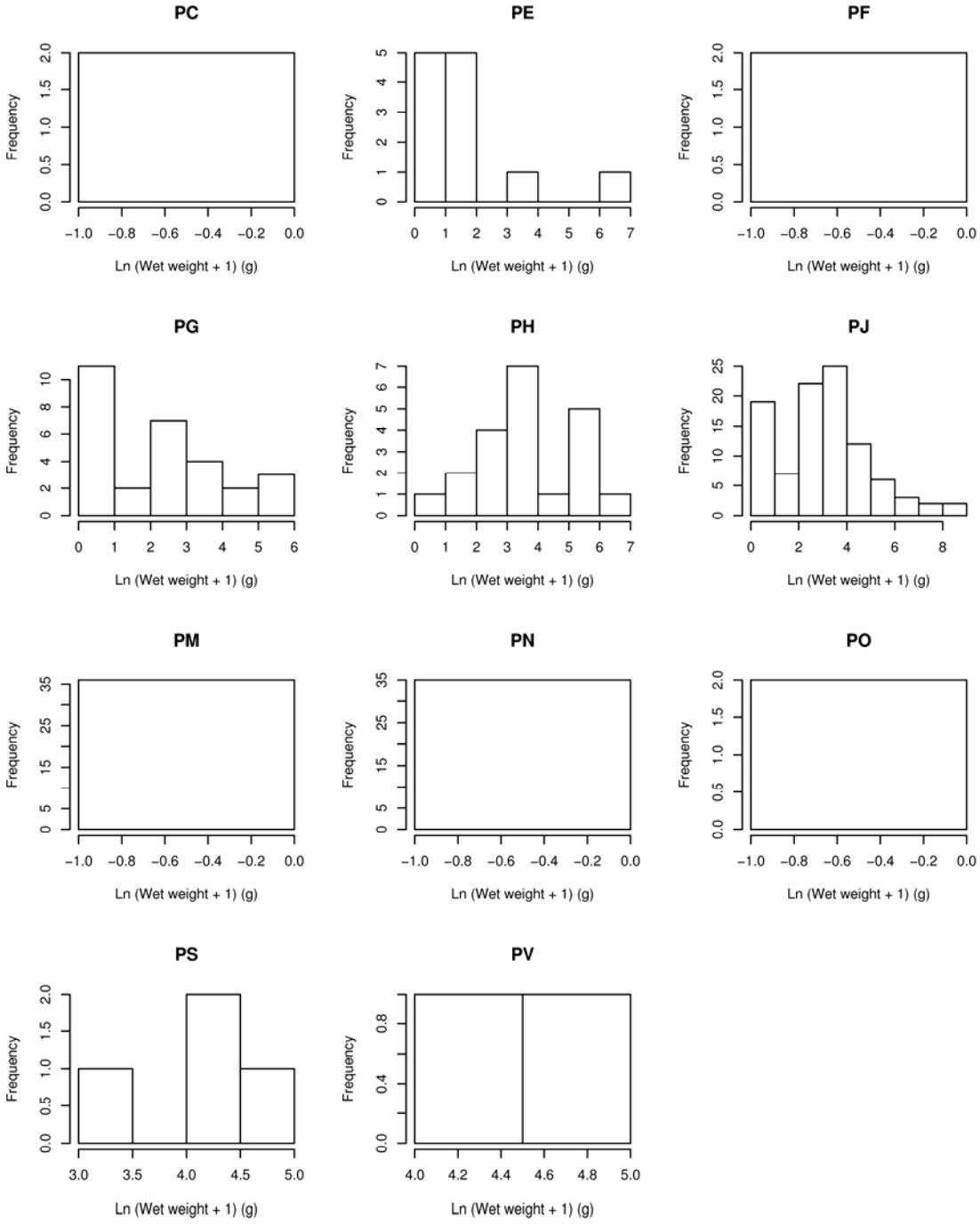



FU
HA
HAA
HAB
HAC
HAF
HAH
HC
HD
HF
HH
Frequency
Ln (Wet weight + 1) (g)

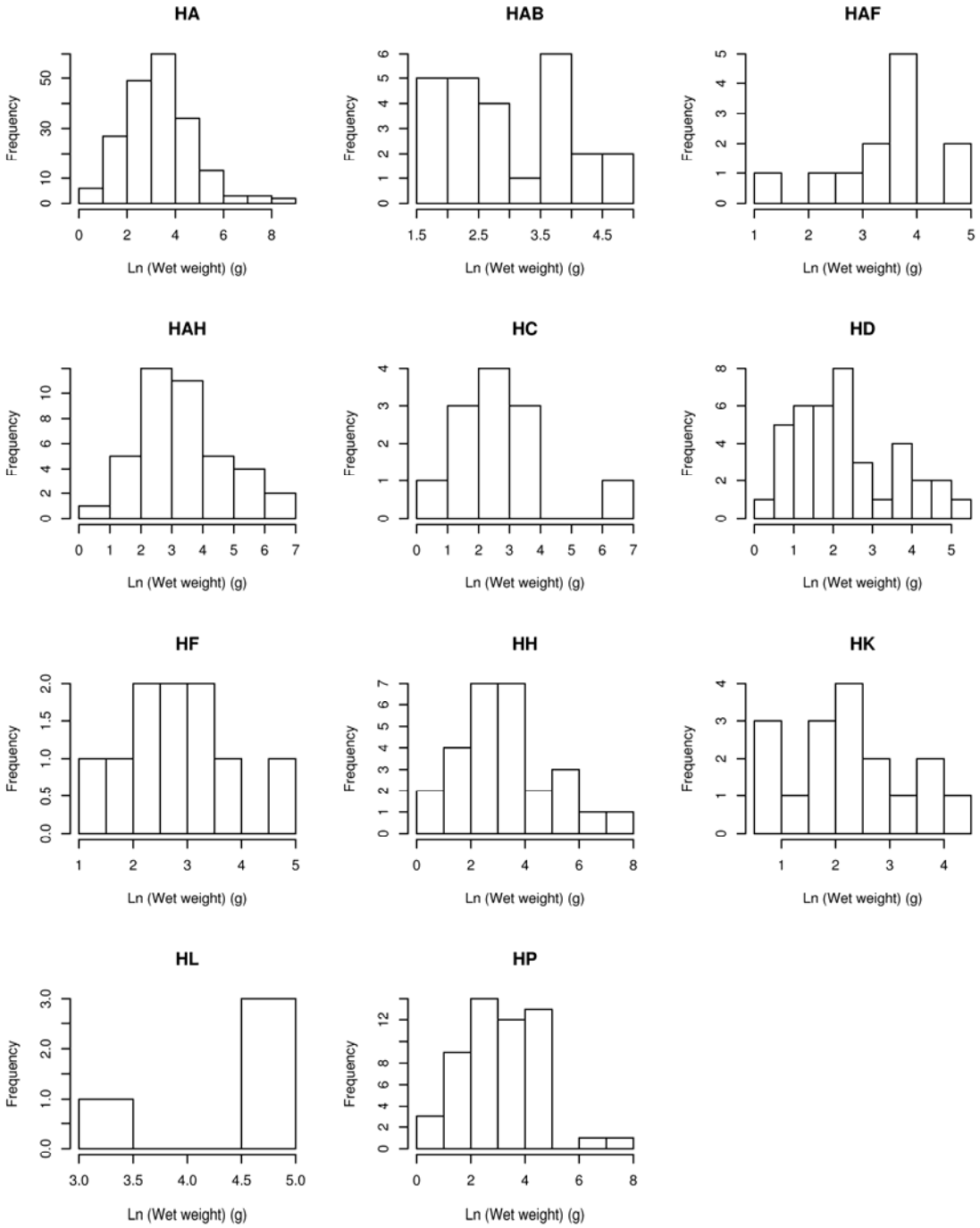

HK
HL
HP
HR
HS
HX
HY
HZ
IA
IB
ID
IF
Frequency
Ln (Wet weight + 1) (g)

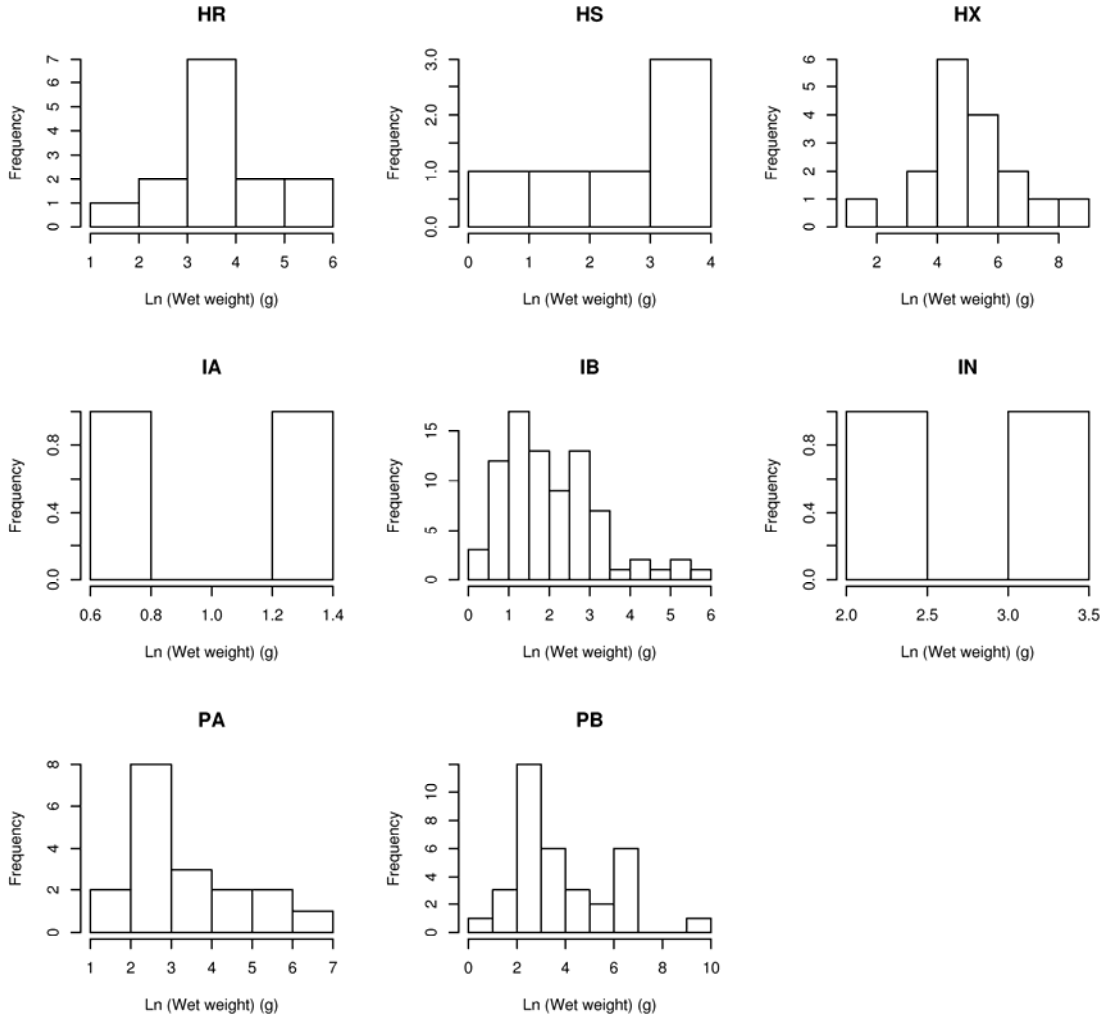

IH
II
IL
IN
IP
IQ
IT
IU
IV
IW
IX
IY
Frequency
Ln (Wet weight + 1) (g)

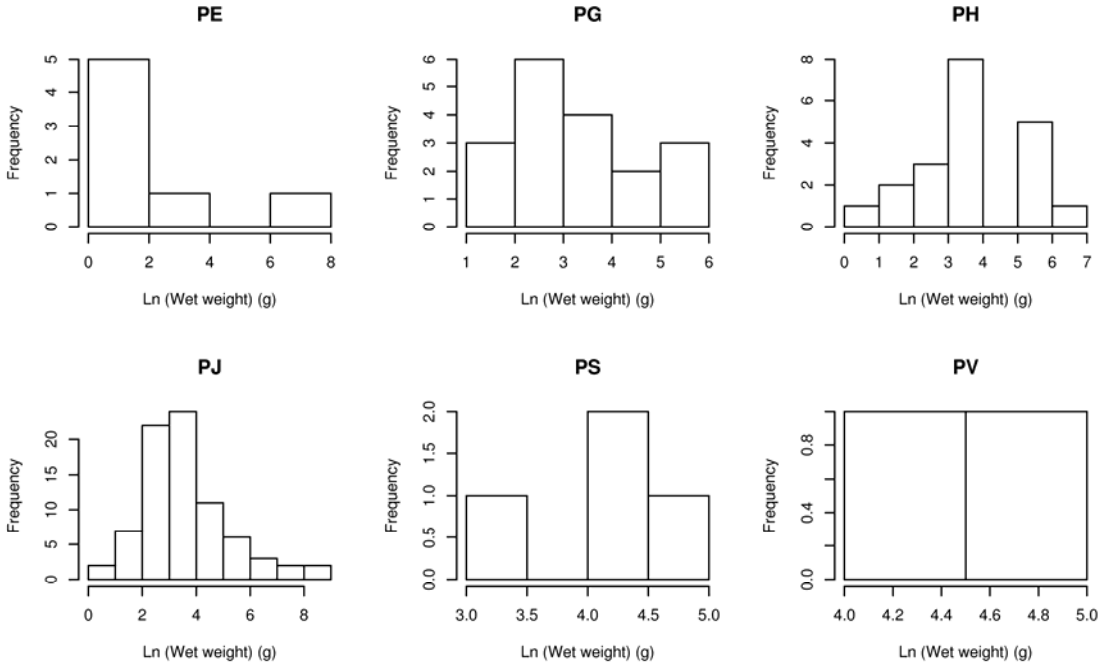

PA
Frequency
0
5
10
15
0 1 2 3 4 5 6 7
Ln (Wet weight + 1) (g)
PB
Frequency
0
5
10
15
20
0 2 4 6 8 10
Ln (Wet weight + 1) (g)


PC
PE
PF
PG
PH
PJ
PM
PN
PO
PS
PV
Frequency
Ln (Wet weight + 1) (g)

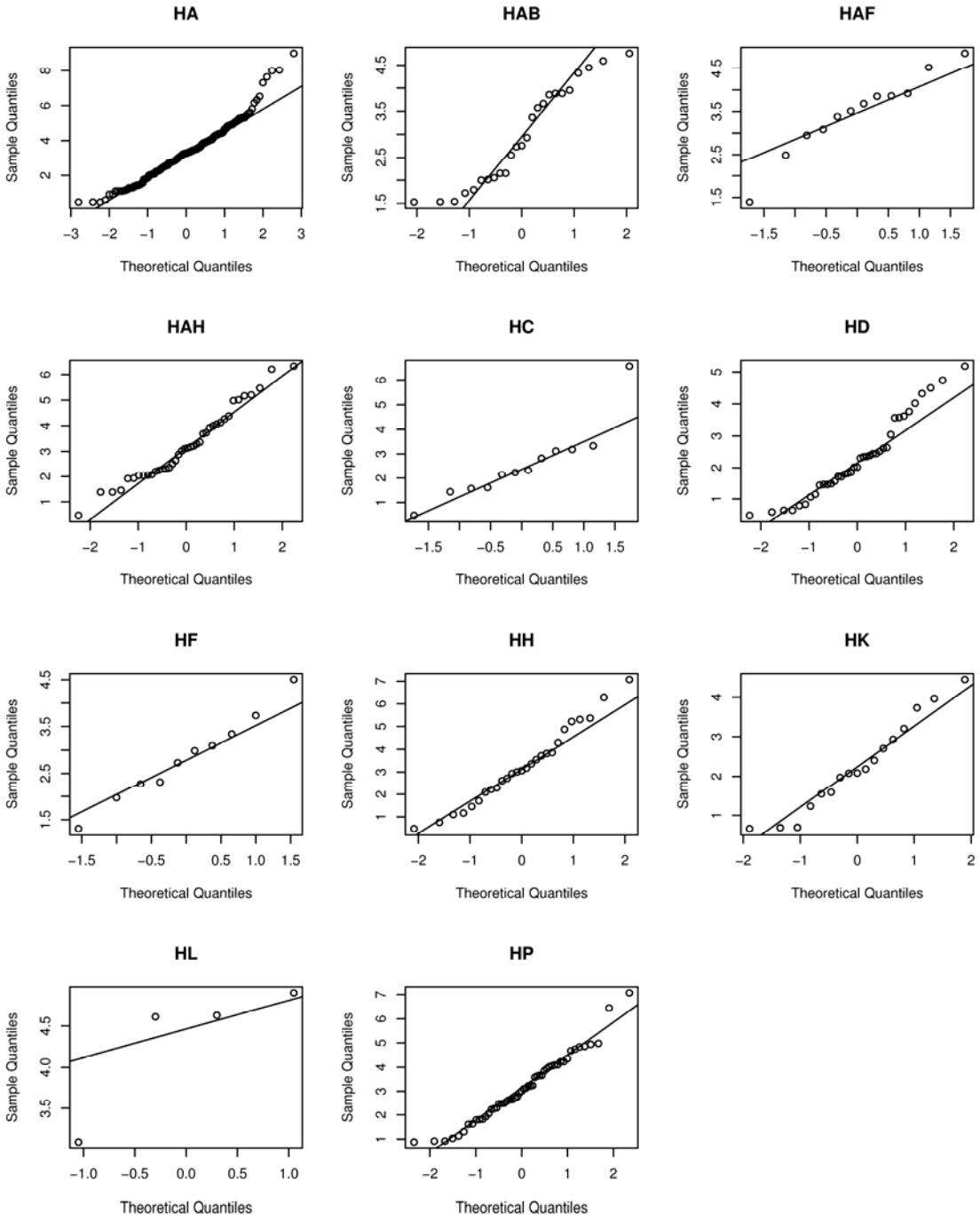
Histograms of the non-zero log-transformed wet weight of fouling biomass by location

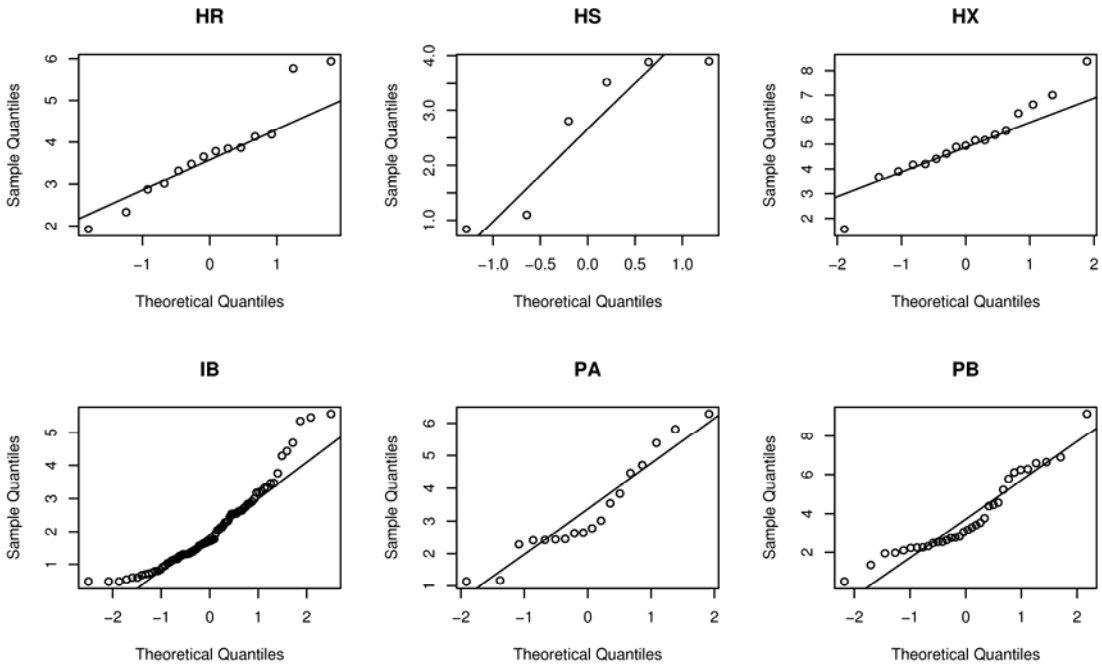


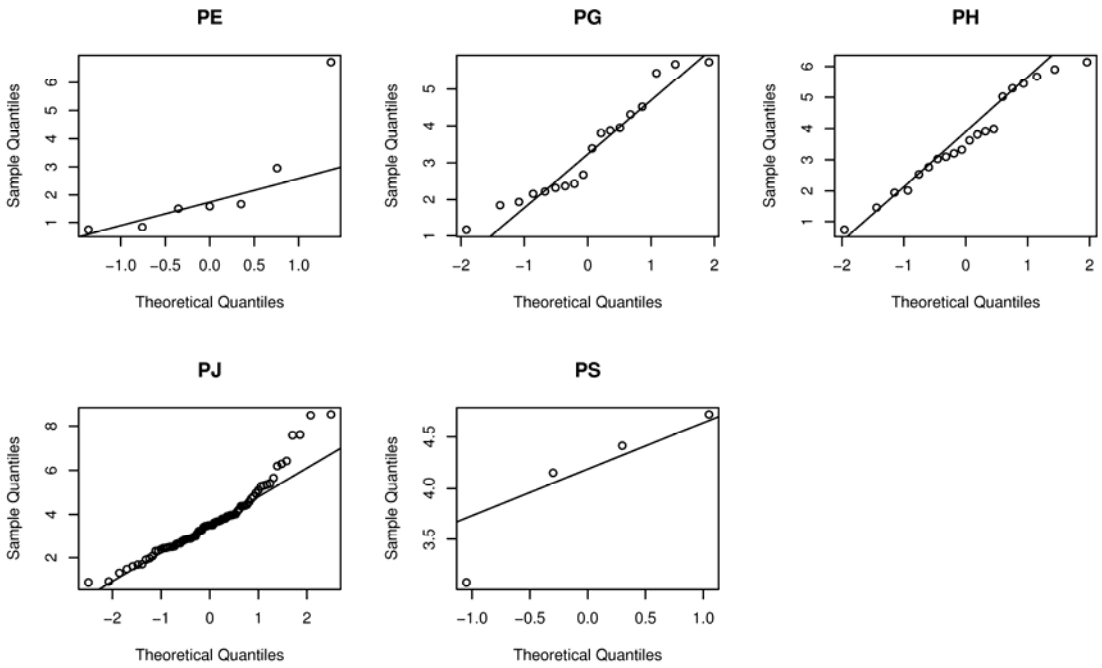

HR
Frequency
Ln (Wet weight) (g)
HS
Frequency
Ln (Wet weight) (g)
HX
Frequency
Ln (Wet weight) (g)
IA
Frequency
Ln (Wet weight) (g)
IB
Frequency
Ln (Wet weight) (g)
IN
Frequency
Ln (Wet weight) (g)
PA
Frequency
Ln (Wet weight) (g)
PB
Frequency
Ln (Wet weight) (g)


PE
Frequency
Ln (Wet weight) (g)
PG
Frequency
Ln (Wet weight) (g)
PH
Frequency
Ln (Wet weight) (g)
PJ
Frequency
Ln (Wet weight) (g)
PS
Frequency
Ln (Wet weight) (g)
PV
Frequency
Ln (Wet weight) (g)

Q-Q plots of the non-zero log transformed wet weight of fouling biomass by location




HR
HS
HX
IB
PA
PB
Sample Quantiles
Theoretical Quantiles


PE
PG
PH
PJ
PS
Sample Quantiles
Theoretical Quantiles

APPENDIX 9 EXAMPLES OF UNDARIA HULL FOULING

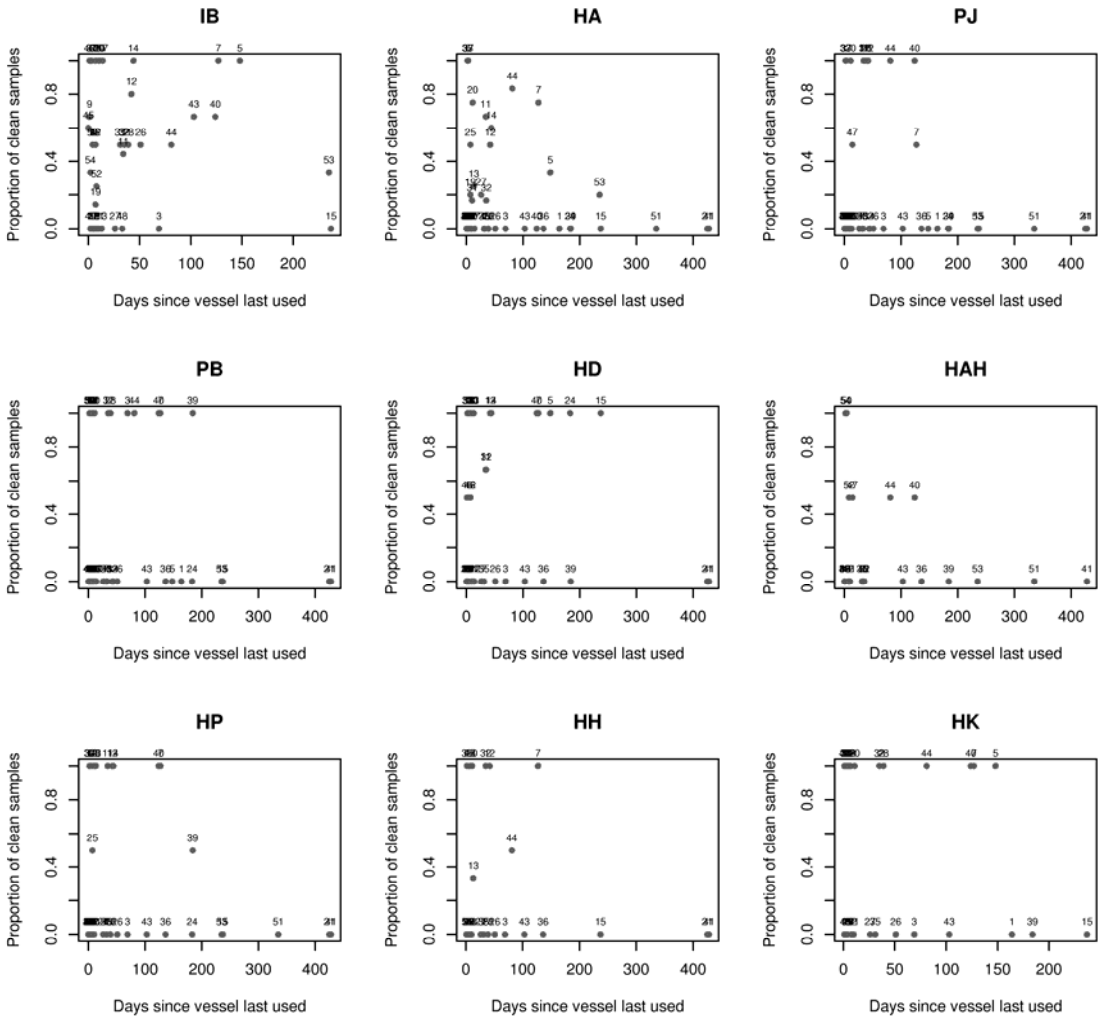
Vessel id: 34

Vessel id: 51

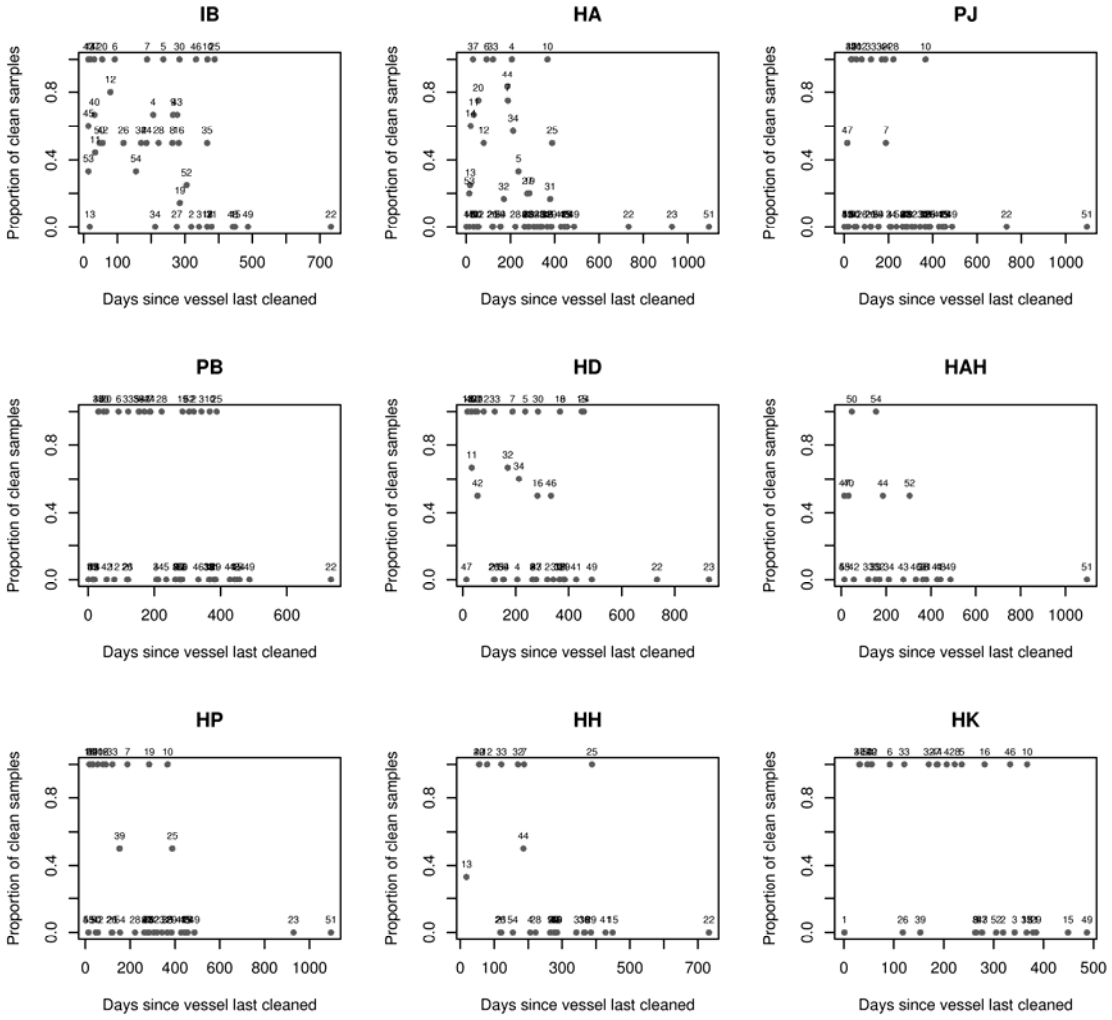
Vessel id: 38

APPENDIX 10 ZERO DATA RELATIONSHIPS

Relationship between proportion of zero-samples and days since vessel last used



Relationship between proportion of zero-samples and days since vessel last cleaned



Relationship between proportion of zero-samples and median trips per annum