OTAGO REGIONAL COUNCIL

Agenda for a meeting of the Technical Committee to be held in the Council Chamber, 70 Stafford Street, Dunedin on Wednesday, 13 September 2017, following the Finance and Corporate Committee

Membership:

Cr Andrew Noone (Chairperson)
Cr Ella Lawton (Deputy Chairperson)
Cr Graeme Bell
Cr Doug Brown
Cr Michael Deaker
Cr Carmen Hope
Cr Trevor Kempton
Cr Michael Laws
Cr Sam Neill
Cr Gretchen Robertson
Cr Bryan Scott
Cr Stephen Woodhead

Apologies:

Leave of Absence:

In attendance:

Please note that there is an embargo on agenda items until 08:30am on Monday, 11 September 2017

CONFIRMATION OF AGENDA

CONFLICT OF INTEREST

PUBLIC FORUM

MINUTES

Minutes of the meeting held on 2 August 2017, having been circulated for adoption.

Otago Regional Council

ACTIONS

Status report of resolutions of the Technical Committee – no current actions to be reported.

PART A RECOMMENDATIONS

Item 1
2017/1019 **Genetic analysis of *Lindavia intermedia*, the diatom the causes lake snow**, DEHS, 29/08/2017

The covering report summarises the background to the Landcare Research report undertaking genetic analyses, the key findings and intended future research work.

The full technical report by Landcare Research entitled "*Lindavia intermedia, the causative organism of New Zealand lake snow: relationship between New Zealand, North American and European populations according to molecular and morphological data"* is circulated separately with the agenda:

PART B ITEMS FOR NOTING

Item 2
2017/0989 **Director's report on progress**, DEHS, 25/08/2017
The report provides information on the: Heavy rainfall event of 21 and 22 July 2017; Clean Water Pacakage 2017 – National Proposed Swimmability Targets; Leith Flood Protection Scheme engineering works;

OTAGO REGIONAL COUNCIL

Minutes of a meeting of the Technical Committee held in the Council Chamber, 70 Stafford Street, Dunedin on Wednesday, 2 August 2017, commencing at 1:45pm

Membership:
Cr Andrew Noone (Chairperson)
Cr Ella Lawton (Deputy Chairperson)
Cr Graeme Bell
Cr Doug Brown
Cr Michael Deaker
Cr Carmen Hope
Cr Trevor Kempton
Cr Michael Laws
Cr Sam Neill
Cr Gretchen Robertson
Cr Bryan Scott
Cr Stephen Woodhead

Apologies: Cr Noone, Cr Laws
The apologies were accepted.

Leave of Absence: Cr Neill

In attendance:
Peter Bodeker (CEO)
Nick Donnelly (DCS)
Fraser McRae (DPPRM)
Michele Poole (Acting DSHE)
Scott MacLean (DEMO)
Sally Giddens (DP&C)
Lauren McDonald (Committee Secretary)
Dean Olsen
Jean Luc Payan

Cr Lawton chaired the meeting in Cr Noone's absence. Mr MacLean in attendance on behalf of the Director of Engineering, Hazards and Science.

CONFIRMATION OF AGENDA
The agenda was confirmed as tabled.

CONFLICT OF INTEREST
Cr Robertson advised of her family connection to the authors of the technical reports attached to Item 2 of the agenda.

PUBLIC FORUM
No public forum held.

Otago Regional Council

MINUTES
Minutes of the meeting held on 14 June 2017, having been circulated were adopted on the motion of Cr Woodhead and Cr Hope. ***Carried.***

ACTIONS
Status report of resolutions of the Technical Committee.

Report No.	Meeting	Resolution	Status
2017/0848 Waiwera River Catchment Water Quality Study	14/6/17	That a stakeholder engagement proposal is brought to the next Communications round.	CLOSED. Item 1 of Communications Committee agenda 2/8/17

PART A ITEMS FOR NOTING

Item 1
2017/0908 **Director's report on progress**, DEHS, 11/07/17
The report provided information on: Lakes Hayes Remediation; review of State of the Environment (SOE) monitoring; Clean Water Package 2017 – National Proposed Swimmability Targets; National Flood Forecasting Model; Sector Research; Waitaki District Council District Plan Review; Leith Flood Protection Scheme; Stock Effluent Disposal Sites.

Discussion was held on the report and included:

Lake Hayes Remediation
Dr Olsen confirmed that ORC have given an undertaking to engage with Friends of Lake Hayes (FOLH) on the Annual Plan target as it progresses. He advised the Dr Schallenberg report for the Friends of Lake Hayes provided four main options:

1. de-stratification (a physical means of stopping the lake stratifying in warmer months);
2. capping the sediments of the lake;
3. Taking water from the Arrow River scheme and placing into Mill Creek to increase the inflow and outflow rate of the lake. This would also take oxygen into lower levels of the lake when it is stratified;
4. Floating wetlands (as remediation)

Mr Bodeker confirmed that as a part of the Annual Plan target for 2017/18 is to work with the community to find a preferred solution. Cost/benefit analysis would be provided through committee reports for agreement, and developed as part of the Long Term Plan (LTP).

State of the Environment Monitoring
- Looking forward to the possible monitoring work Council may wish to focus on for freshwater, land and air etc.
- A technical review of reporting for:
  a) What Council are required to monitor (SOE) and b) what Council wish to monitor (targeted) could be considered.
- The value of long term monitoring for the Council to inform and understand the complex systems in regard to policy planning.

Clean Water Package 2017 – National Proposed Swimmability Targets

It was confirmed there are four assessments metrics required for the proposed 'swimmability' attribute states:

- percentage of exceedances over 540 E.coli per 100ml
- Median E.coli per 100ml
- 95th percentile E.coli per 100ml
- Percentage of samples above 260 E.coli per 100ml.

Dr Olsen confirmed that Schedule 15 was the Council measurement of success for the metrics and was well aligned for the Clean Water Package.

A concern was raised in regard to the presentation of complex information in the report.

Action: The section of the Director's report on the Clean Water Package to be re-written in a clearer format and re-presented to the Technical Committee.

Leith Flood Protection Scheme

A question was raised in regard to NZTA funding for the Dundas Street stage of the scheme. Mr Bodeker confirmed discussion would be held with DCC and the NZTA.

Moved Cr Lawton
Seconded Cr Deaker

That the report is noted.

Motion carried

Item 2
2017/0940 **Ecological Assessment of the Waikouaiti, Catlins and Shag Estuaries**, DEHS, 17/07/17

The covering report summarised the 2016/17 ecological assessments carried out in the Waikouaiti, Catlins and Shag Estuaries to inform the development of the Coastal Strategy and to complement work being undertaken on the effects of surface water-groundwater interactions on water quality in the lower Shag River.

The technical reports: *Waikouaiti Estuary - Broad Scale Habitat Mapping 2017/17; Waikouaiti Estuary - Fine Scale Monitoring 2016/17; Catlins Estuary - Broad Scale Habitat Mapping 2016/17; Catlins Estuary - Fine Scale Monitoring 2016/17; Shag Estuary - Broad Scale Habitat Mapping 2016/17; Shag Estuary - Fine Scale Monitoring 2016/17* were circulated separately with the agenda.

Cr Robertson's declared her family connection to the authors of the technical reports tabled. This was noted and not considered as a conflict of interest.

Dr Olsen summarised the covering report and confirmed ecological monitoring on other estuaries was still to be undertaken for the Otago Harbour and Blueskin Bay. The Tokomariro Estuary and the Kaikorai Estuary were included in the work programme for the current financial year 2017/18. He confirmed that scoping of the ecological assessment work was currently underway and would be completed by external contractors.

Moved Cr Lawton
Seconded Cr Bell

That this report and the Technical reports and attachments (as listed) are received and noted.

Motion carried

Cr Scott took the opportunity to acknowledge and thank staff for their positive work in regard to the flood protection schemes during the July flood event, which had been a credit to Council.

The meeting was declared closed at 2:26pm.

Chairperson

Otago Regional Council

REPORT

Document Id: A1026025

Report Number: 2017/0989
Prepared For: Technical Committee
Prepared By: Dr Jean-Luc Payan, Manager Natural Hazards
Dr Dean Olsen, Manager Resource Science
Chris Valentine, Manager Engineering
Date: 25 August 2017

Subject: **Director's Report on Progress**

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1. 21st and 22nd July 2017 Heavy Rainfall Event

Staff are compiling the information needed to comprehensively report on the heavy rainfall event that occurred between 21 and 22 July and associated observations (weather pattern, severity of the event, hydrology, flows and water levels, etc.) and consequences (inundation extents, landslides, etc.). A series of surveys, observation collection and analysis are being prepared to inform the report. This includes:

- An extensive flood debris mark survey on the main rivers and streams on the Taieri Plain. This data is critical to capture as it is will be used to assess the performance of the Lower Taieri Flood Protection Scheme and to inform future investigations associated with the Scheme;
- Debris marks surveys in other areas in the region where justified by the severity of the event and where work is planned such as on the Waitaki Plain between Pukeuri and the Waitaki River or on the Water of Leith;
- Inspections of waterways and the coastline in the Otago Region;
- A report describing the weather pattern. NIWA has been engaged to analyse the weather conditions that resulted in the heavy rainfall event and to compare it to historical rainfall events such as the June 1980 event. This will help understanding the spatial and temporal variability of the July 2017;
- The creation of a database of photographs and videos taken during the event. The database contains over 3,000 photographs and videos captured by the ORC and other organisations involved in the response to the event. Satellite images showing the extent of flooding have also been included;
- The localisation and mapping of over 400 landslides that were triggered by the heavy rainfall event. Those landslides are in addition to the ones investigated by the Dunedin City Council.

The July event was a significant test for the Lower Taieri Flood Protection Scheme however the scheme generally operated well. Localised overtopping occurred on the Contour Channel, right bank of the Silver Stream (flow to upper pond from the Gordon Road spillway), and East Taieri Upper Pond cut off bank. Riverside Road Spillway manual gates were lowered late Friday night reducing peak flow downstream in the Taieri River. Overall the scheme directed and contained flood waters as designed.

Seepage of water between the Mill Creek pumping station and the adjacent floodbank began to undermine the integrity of floodbank in this location early on 22 July (Figure 1). Failure of the floodbank in this location could have caused inundation within the East Taieri Lower Pond to beyond Gladfield Road, and area of approximately 1600 hectares. Engineering and Operations staff along with the assistance of contractors and the New Zealand Army were able to stem the flow using sandbags to seal the floodbank on the true left of the pump station. Tonkin and Taylor have been commissioned to design a temporary repair, which has been completed (Figure 2), and undertake a forensic investigation of the cause of the seepage and piping risk. The scope of the floodbank integrity assessment work has been increased to now include more detailed investigation and analysis of all large ORC structures keyed into earthen floodbanks.

Figure 1: **Seepage flow around Mill Creek pump station (East Taieri Drainage Scheme) on 22 July 2017**

Figure 2: **Mill Creek pump station (East Taieri Drainage Scheme) with temporary repairs underway to reduce risk of further seepage and piping**

All floodbanks in the scheme were inspected once safe to do so, these inspections started at first light on 22 July. The floodbank inspections were prioritised by likelihood and consequence of failure/damage. All initial inspections were completed by 24 July. Post event recovery is still ongoing; actions are being prioritised in order of risk. Investigations such as options to improve power supply reliability for the Waipori pump station are being scoped for incorporation into the 2018/28 Draft Long Term Plan.

The community response to the event was generally positive with over 50 issues raised by the community in relation to Taieri Schemes and Council managed Dunedin urban waterways. Approximately 90% of these issues have been closed at time of writing with the remaining still being addressed by staff.

2. Clean Water Package 2017 - National Proposed Swimmability Targets

The government has recently amended the 2014 National Policy Statement for Freshwater Management (NPSFM). It sets national targets relating to 'swimmability' for New Zealand's rivers and lakes. The Clean Water Package includes numerous other changes to the NPSFM such as provisions for stock exclusion, and requirements for regional councils to monitor the ecological health of our rivers and lakes. The changes can be viewed online at the MfE website [1].

There are many factors that can affect whether a water body is suitable for swimming, such as water clarity, but the government has decided that swimmability will be assessed on potential health risks. This will be determined by *E. coli* concentrations (faecal indicator bacteria sourced from warm-blooded animals) in rivers; and toxic algae bio-volumes in lakes. A swimmable river is one with low levels of *E. coli* and a swimmable lake is one with low levels of toxic algae.

The Government has set a national target of making 90 percent of New Zealand's rivers (fourth order or greater) and lakes (with perimeters greater than 1.5 km) swimmable by 2040. The stream order describes the relative size of streams. Streams with no tributaries are "first order", streams with two first order tributaries are second order, and with two second order tributaries are third order and so on. Examples of fourth order streams in the Dunedin locale include the Water of Leith alongside the University of Otago, Silverstream at Mosgiel and the Kaikorai Stream at State Highway 1. The Manuherikia River at Alexandra is seventh order and Otago's biggest river, the Clutha at Balclutha, is eighth order. Around 90 percent of New Zealand's catchments flow into rivers that are fourth order or bigger (MfE website).

The NPSFM grading proposals are based on a "sophisticated" grading system that uses four statistical measures of *E.coli* concentrations when assessing river swimmability; and one statistical measure for toxic algae bio-volumes when assessing lake swimmability.

The government received numerous submissions stating that the grading system for *E. coli* in rivers is overly complex. However, in the gazetted changes to the NPSFM the government has retained all four tests when assessing river swimmability. The four statistical measures of river *E.coli* data are presented in Table 1. As stated in the footnote to Table 1, "Attribute state must be determined by satisfying all numeric attribute states".

The suggested changes to the NPSFM also require regional councils to develop regional targets to contribute to the national target. They must make draft regional targets available to the public by March 2018, and make their final regional targets public by the end of 2018.

[1] https://www.mfe.govt.nz/sites/default/files/media/npsfm-showing-changes_0.pdf

Otago Regional Council has set targets for *E. coli* for the region in the Regional Plan: Water under Schedule 15 (Table 2). Staff have compared *E. coli* data collected throughout the region from the State of Environment monitoring network and developed relationships to assess compliance with Schedule 15 limits and the 4 separate statistical tests within the NPSFM. This work has shown:

- That the *E. coli* limits set in Schedule 15 for water catchment Group 3 (Upper Clutha upstream of the Southern Great Lakes) provides compliance against the four separate statistical tests in the NPSFM and as a minimum, will provide a blue (A grade) or green (B grade) swimmability category. The minimum requirement is an orange or C grade.

- With the exception of some catchments in the Pomahaka, the *E. coli* limits set in Schedule 15 for water catchment Groups 1 and 2 (that covers the remainder of the Otago region), will provide good compliance against the four separate statistical tests in the NPSFM, and as a minimum, will provide a blue (A grade), green (B grade) or in some cases an orange (C grade) category. The Orange, C grade category being the minimum requirement.

- In the case of the Pomahaka catchment, monitoring sites in some catchments return high 95th percentiles at all flows (Table 1) even though they may be compliant with the Schedule 15 limit (Table 2). The reason for this is thought to be due to effluent storage issues and mole and tile drains resulting in very high *E. coli* peaks under high flow conditions. ORC are working actively throughout the Pomahaka catchment with groups such as the Pomahaka Watercare Trust, the Landcare Trust and the Clutha Development Trust to address water quality issues. A large part of this effort is focused on improving bacterial water quality.

A report is being prepared by MfE that will outline where regions currently sit in regards to swimmability. The report is being informed by information being provided by regional councils including ORC. It will outline mitigation measures that regional councils and territorial authorities are currently committed to that will improve swimmability. It is expected that this report will be available early November, 2017.

Otago Regional Council

Table 1. **The *E. coli* swimming categories (attribute states) in detail. Taken directly from the MfE websites at http://www.mfe.govt.nz/freshwater/national-targets-swimming-water-quality/water-quality-swimming-categories-attribute.**

Category	Percentage of exceedances over 540 *E.coli* per 100 ml	Percentage of samples above 260 *E.coli* per 100 ml	Median: *E.coli* per 100 ml	95th percentile: *E.coli* per 100 ml	Narrative risk descriptor
What it means	How often the river exceeds the acceptable threshold for swimming	How often the river goes over the point where additional monitoring is needed at primary contact sites	The mid-point (ie, half the time *E. coli* is lower than this, half the time it is higher)	*E. coli* only rarely goes past this point (only 5% of the time)	Risk of Campylobacter infection (based on *E.coli* indicator)
A (Blue)	<5%	<20%	≤130	≤540	For at least half the time, the estimated risk is <1 in 1000 (0.1% risk) The predicted average infection risk is 1%*
B (Green)	5-10%	20-30%	≤130	≤1000	For at least half the time, the estimated risk is <1 in 1000 (0.1% risk) The predicted average infection risk is 2%*
C (Yellow)	10-20%	20-34%	≤130	≤1200	For at least half the time, the estimated risk is <1 in 1000 (0.1% risk) The predicted average infection risk is 3%*
D (Orange)	20-30%	>34%	>130	>1200	20-30% of the time the estimated risk is ≥50 in 1000

					(>5% risk) The predicted average infection risk is >3%*
E (Red)	>30%	>50%	>260	>1200	For more than 30% of the time the estimated risk is ≥50 in 1000 (>5% risk) The predicted average infection risk is >7%*

* The predicted average infection risk is the overall average infection to swimmers based on a random exposure on a random day, ignoring any possibility of not swimming during high flows or when a surveillance advisory is in place (assuming that the *E.coli* concentration follows a log-normal distribution). Actual risk will generally be less if a person does not swim during high flows.

1 Attribute state should be determined by using a minimum of 60 samples over a maximum of 5 years, collected on a regular basis regardless of weather and flow conditions. However, where a sample has been missed due to adverse weather or error, attribute state may be determined using samples over a longer timeframe.

2 Attribute state must be determined by satisfying all numeric attribute states.

Table 2: Regional Plan Water Schedule 15 limits for *E. coli* for the different surface water catchment groups that apply to rivers.

Schedule 15	**80th percentile *E. coli* concentration when flows are below median flow**
Group 1	260
Group 2	260
Group 3	50

3. Leith Flood Protection Scheme

Engineering works on the Union to Leith Footbridge stage of the Scheme are progressing. The majority of the right bank wall extension has been completed (Figure 3).

Figure 3: **Leith Flood Protection Scheme works underway on right bank between Union Street and Leith Footbridge on 21 August 2017 (top) and 24 August 2017 (bottom).**

Left bank widening upstream of the University of Otago ITS building is nearly complete. Slope stability issues have arisen in relation to the temporary cut due to unforeseen ground conditions. Significant temporary works have been undertaken in this location to ensure the stability of the bank and University assets and maintain a safe work space for the construction of the new left bank wall (Figure 4). The stability of the ITS building is not affected.

Figure 4: **Leith Flood Protection Scheme works underway on left bank between Union Street and Leith Footbridge on 24 August 2017**

As previously advised to committee, some of the construction works will extend beyond the planned completion date due to the discovery of asbestos, the weather events in April and July and other factors. Whilst the contractor anticipates that there will still be some siteworks continuing into early 2018, most of the works will be completed by the end of this calendar year with the remainder of the works happening on the river bed near the downstream end of the site. Parts of the site will be handed back and site fencing removed as packages of work are completed later this year. Staff are continuing to liaise closely with University of Otago Property Services so as to minimise disruption to students, staff and visitors and with University communications staff to ensure that the University community has regular updates.

Investigations for the Dundas Street stage of the Scheme are continuing. A civil and structural design contract for the culvert and modification to the existing bridge has been awarded to Opus Consultants Ltd. Following the initial options/feasibility workshop, a preferred option to replace the bridge with a series of three box culverts to widen the channel was identified and is being developed. This option has a number of constructability and program benefits.

4. Recommendation

That this report is noted.

Gavin Palmer
Director Engineering, Hazards and Science

Otago Regional Council

REPORT

Document Id: A1030016

Report Number: 2017/1019
Prepared For: Technical Committee
Prepared By: Dean Olsen, Manager Resource Science
Date: 29 August 2017

Subject: **Genetic analysis of *Lindavia intermedia*, the diatom that causes lake snow**

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1. Précis

Landcare Research was commissioned by Otago Regional Council (ORC) to undertake genetic analyses to determine whether the diatom responsible for forming lake snow, *Lindavia intermedia*, is a recent arrival in New Zealand. These analyses compared samples from overseas lakes to samples collected from a number of New Zealand lakes using advanced genetic techniques. Samples from New Zealand lakes included material from Lakes Coleridge, Hawea, Wakatipu and Wanaka while samples were obtained from two lakes in North America: Lake Youngs in Washington State and Cultus Lake in British Columbia, and one lake in Europe – Lac Leman on the border of Switzerland, France and Italy. Attempts to secure material from other localities were unsuccessful.

The genetic analysis involved the Landcare team identifying areas of high genetic variability in the chloroplast and nuclear material of *L. Intermedia* and developing genetic primers[1] specifically to compare the genetic similarity between the different localities.

The key finding was that specimens from Lake Youngs (USA) and all New Zealand lakes were genetically and morphologically identical. This strongly suggests that *Lindavia intermedia* is not a New Zealand native species and that its most likely origin is North America.

If *Lindavia* was native to New Zealand, it would be expected to exhibit some genetic variability from lake to lake, due to genetic isolation and evolution of the populations in each of the lakes. For example, samples from Cultus Lake and Lake Youngs, both of which are in North America, exhibited some genetic differences in this analysis. If *Lindavia* was native to New Zealand, it would also be expected to be genetically distinct from Northern Hemisphere populations due to the long physical distance between these populations and the (presumably) restricted exchange of material between these populations that would prevent genetic divergence (separation) over time. It is also worth noting that there are no known populations of *Lindavia intermedia* in other Southern Hemisphere lakes.

[1] A primer is a short strand of RNA or DNA (generally about 18-22 bases) that serves as a starting point for DNA synthesis.

Taken together with the results of sediment coring in New Zealand lakes, the results of the genetic analyses undertaken by Landcare Research represent a strong circumstantial case for transfer of *Lindavia intermedia* to New Zealand from the Northern Hemisphere, particularly North America.

2. Where to from here?

During the workshop with experts late in 2016, a research plan was developed, prioritising research needed to inform and support management of lake snow. This plan has previously been presented to Council (2017/0705, 15 March 2017; Report number 2017/0802, 14 June 2017) and is attached to this report (Appendix A). The development of the plan occurred alongside an understanding that *Lindavia intermedia* was, in all likelihood, not a New Zealand native species. The research plan priorities are still relevant in light of the findings of the Landcare Research genetics work commissioned by ORC.

The Science team of ORC are working with Otago University and Landcare Research to progress scoping and delivery of the key research components (Components 1 ii); 1 iii); 2A i); 2B i); and 5) identified in Appendix A in line with the 2017/18 ORC Annual Plan. The Annual Plan makes financial provision for these key research components[2].

The identification of appropriate ways of managing lake snow in the Southern alpine lakes will require ORC to work collaboratively with Ministry of Primary Industries, Environment Canterbury and Environment Southland. That collaboration commenced with their participation in the experts' workshop convened by ORC in 2016. Arrangements are being made to start the next phase of that collaboration.

3. Recommendations

1. The report "*Lindavia intermedia*, the causative organism of New Zealand lake snow: relationships between New Zealand, North American and European populations according to molecular and morphological data" is received and noted.

2. The actions being taken by Otago Regional Council in response to the findings in that report are noted.

3. That Council will continue to work collaboratively with stakeholders and interested parties to develop feasible methods of managing the effects of lake snow on water quality

Gavin Palmer
Director Engineering, Hazards and Science

[2] The 2017/18 Annual Plan includes the target "*Continue to lead research into feasible methods of managing the effects of lake snow on water quality*".

Otago Regional Council

Appendix A

Table 1. **Research priority work streams, priority ranking, associated costs and justification.** ***The table below complements the summary table provided in the proceedings of the 20 December 2016 experts' workshop.***

Priority Ranking	Code
High - Immediate	**High - Immediate**
High - Medium term	**High - Medium term**
Medium - Medium term	**Medium - Medium term**

Work stream	Sub-program	Priority Ranking	Associated costs	Justification	Lead agency
1) Is *Lindavia intermedia* a native or non-native species? ***Top priority area. Will influence the direction of other work streams***	i) Investigation of cell genetics (microsatellite analysis) of NZ and overseas *L. intermedia* populations	**High - Immediate**	***Currently funded by ORC. To be delivered by end of Jun 17.***	This work will indicate if *L. intermedia* has recently arrived in NZ and should be considered an invasive species.	**ORC**
	ii) Comprehensive examination of NZ diatom samples, collections, reports	**High - Immediate**	**$11K** for detailed assessment of 3 separate catalogued collections Delivery 3 to 6 months.	To determine if previous '*Cyclotella*' identifications are in fact *Lindavia*. To help isolate the length of time the diatom has been present in NZ.	**ORC**
	iii) Historical dynamics of *L. intermedia* in NZ lakes from which it has been reported using paleolimnological diatom analysis of dated sediment cores.	**High - Immediate**	4 priority lakes in Otago **$56K.** ($14K per lake). Delivery 6 to 9 months for Otago's 4 priority lakes.	This work will allow a precise estimate of the time that *L. intermedia* has been present in NZ and will complement the microsatellite work currently being undertaken in (i) above.	**ORC**

			Estimated 10 lakes needed to be cored across Otago, Southland, Canterbury and Hawke's Bay		
2) What are the drivers of: **(A) *L. intermedia* dominance in lakes and**	2A i) Literature review of shifts in lake phytoplankton to increased dominance by (*Lindavia*-like) centric diatoms (e.g., climate connection)	**High - Immediate**	$3K – if aligned with 2B i).	This would increase our understanding of shifts and drivers of phytoplankton community structure to one dominated by centric diatoms and provide extremely valuable information to the NZ context.	**ORC**
	2A ii) Are historical *L. intermedia* dynamics correlated to environmental drivers in our lakes?	**Medium - Medium term**	$219K Delivery 3 years [Note: This work is covered in the University of Otago MBIE bid.]	As with 2B ii) this work-stream is extensive and likely best delivered through a University and a number of postgraduate and post-doctoral research programs.	**Catchments Otago / Uni. Of Otago / CRIs / support from RC's**
	2A iii) Are proliferations of *Didymo* and *L. intermedia* in South Island waters related to	**Medium - Medium term**	$19K minimum Delivery difficult to estimate	If the timing and spread of these two incursions are coherent, then that would provide evidence	**Catchments Otago / Uni. Of Otago / CRIs / support from RC's**

	a common driver or species incursion?			of a common incursion (both place and time) and support management of future incursions and responses.	
2) What are the drivers of: (B) polysaccharide overproduction by *L. intermedia*?	2B i) Comprehensive literature review on diatom polysaccharide overproduction from similar situations overseas	**High - Immediate**	$10K Delivery 3 to 6 months	Seen as a top priority and would increase our current understanding of TEP production and the lake snow phenomenon. A straightforward exercise that hasn't been undertaken to date.	**ORC**
	2c) Study of the relationships between diatom polysaccharide overproduction and (1) nutrient availability, (2) climate warming, and (3) grazing pressure.	**High - Medium term**	Year 1: $204K Year 2: $211K Year 3: $198K Delivery 3 years [Note: This work is covered in the University of Otago MBIE bid.]	As with 2A ii) this work-stream is extensive and likely best delivered through a University and a number of postgraduate and post-doctoral research programs.	**Catchments Otago / Uni. Of Otago / CRIs / support from RC's**
3) Can we develop technologies for effective sampling and monitoring of	i) The development of new sensor technology to monitor in situ polysaccharide concentrations in	**High - Medium term**	$300K per year for three years - Part of an MBIE Smart Ideas bid – decision on	Capacity to monitor the abundance and spatial variability of lake snow is critical to	**Landcare Research / Uni. Of Otago / Support from ORC**

***L. intermedia* and lake snow?**	lakes.		success due Sept 2017.	understanding the environmental drivers that lead to lake snow production. At present these techniques do not exist.	
	ii) The development of cost-effective and efficient methods for quantitatively sampling lake snow in lakes (at different depths).	**High - Medium term**			**Landcare Research / Uni. Of Otago / Support from ORC**
	iii) Can DNA methods be developed for the sensitive detection of *L. intermedia* in lakes?	**Medium - Medium term**			**Landcare Research / Cawthron / support from RC's**
4) How might the spread of *L. intermedia* between lakes be stopped or slowed?	i) Are the BNZ Didymo sanitation methods adequate for the disinfection of *L. intermedia*?	**High - Immediate**	Currently contracted by MPI who have engaged NIWA to review the effectiveness of Check – Clean – Dry on *Lindavia*	MPI are reviewing their Check/Clean/Dry campaign and how effective it is for other pest species.	**MPI / NIWA**
5) Supporting citizen science		**High - Medium term**	$10K	Links to 3.	**ORC**

LANDCARE RESEARCH
MANAAKI WHENUA

Lindavia intermedia, the causative organism of New Zealand lake snow: relationships between New Zealand, North American and European populations according to molecular and morphological data

Lindavia intermedia, the causative organism of New Zealand lake snow: relationships between New Zealand, North American and European populations according to molecular and morphological data

Phil Novis, Caroline Mitchell and Ana Podolyan

Landcare Research

Prepared for:

Otago Regional Council

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August 2017

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Reviewed by:	*Approved for release by:*
	
Rob Smissen Research Priority Area Leader, Plants Landcare Research	Justine Daw General Manager Landcare Research

Landcare Research Contract Report: LC2991

Disclaimer

This report has been prepared by Landcare Research for Otago Regional Council. If used by other parties, no warranty or representation is given as to its accuracy and no liability is accepted for loss or damage arising directly or indirectly from reliance on the information in it.

Contents

Summary

Project and client

- Otago Regional Council contracted Landcare Research to undertake an investigation into the genetics of *Lindavia intermedia* populations in New Zealand and overseas, including the development and implementation of new molecular markers suitable for determining intraspecific variation, and testing hypotheses of the origin of this species in New Zealand.

Objective

- To test whether *Lindavia intermedia*, the causative agent of lake snow in New Zealand, is a recent invader in New Zealand lakes.

Methods

- DNA extracted from *L. intermedia* sourced from three New Zealand lakes was subjected to shotgun genome sequencing using Illumina Miseq.
- Assembled sequences were used to generate potential new molecular markers as tools to address the research question.
- These markers were tested and screened on New Zealand material.
- Selected markers were applied to samples from New Zealand, North America (two sites) and Europe (one site).
- Genetic diversity and phylogeographic relationships among populations of the species from these three areas were examined.

Results

- Three new nuclear markers and three new chloroplast markers were regarded as robust and combined to generate new data from the study populations.
- Specimens from Lake Youngs (USA) and all New Zealand lakes tested were identical, both genetically and morphologically. Specimens from Cultus Lake (Canada) were distinct genetically according to all markers, and were slightly different according to morphological data. The Lac Léman sample was the least similar to the New Zealand material.

Conclusions

- Although limited by the number of samples available from overseas, the results in context provide strong circumstantial evidence for the recent invasion of New Zealand lakes by *L. intermedia*.

Recommendations

- The status of *L. intermedia* as an invasive organism in New Zealand is now fairly certain. However, further study is needed to determine management options, its history in different New Zealand lakes and its effect on lake ecology, and to resolve the long-outstanding taxonomic issues surrounding bodanicoid *Lindavia* species (for which the newly developed markers offer useful tools). Such studies require funding at a national level.

1 Introduction and objectives

In 2008 Dr Marc Schallenberg and his students from Otago University began to study the production of an unusually abundant slime occurring in Lake Wānaka. Marc identified this as the globally rare phenomenon known as 'lake snow' (so called due to the drifting appearance of the flocs when viewed underwater; Brachvogel et al. 2001; Grossart et al. 1997). The abundance of this slime has been significant enough to require upgrades to municipal water infrastructure, and it has proven disruptive to recreational and professional fishing on the lake (Williams 2017).

The same phenomenon was identified in Lake Coleridge by Landcare Research in 2015 (although it was known to local bach owners about a year beforehand), and the mucilaginous strands were found to be produced by the same species in both cases. This species was identified as the diatom *Lindavia intermedia* (Manguin ex Kociolek & Reviers) Nakov, Guillory, Julius, Theriot & Alverson ex W.C.Daniels, Novis & Edlund (Novis et al. in press), a name that required taxonomic validation (Daniels et al. 2016). Lake snow was discovered in Lake Wakatipu and Lake Hawea shortly thereafter, and water filtration issues similar to those at Wānaka became evident in Queenstown (Scott 2017).

During this apparent range expansion in New Zealand, Schallenberg and Novis coined the vernacular term 'lake snot' as a result of its appearance on fishing lines and filters, and in reference to *Didymosphenia geminata* (Lyngbye) Schmidt, an invasive diatom in clean New Zealand rivers that is sometimes known as 'rock snot'. Although not used to date in the scientific literature, the term 'lake snot' has become popular in the news media. Both 'lake snow' and 'lake snot' refer to the same phenomenon.

Historical cases of slime outbreaks in New Zealand lakes were re-examined on the basis of this new knowledge. A 2008 event in Lake Waikaremoana (North Island) was definitively shown to have been due to the same organism, thanks to a preserved specimen held at NIWA by Dr Cathy Kilroy. Another occurrence in Lake Benmore during the early 2000s was caused by an organism identified as *Cyclotella stelligera* (Cleve & Grunow) van Heurck (now *Discostella stelligera* (Cleve & Grunow) Houk & Klee). This species could certainly be confused with *L. intermedia*, but this is currently impossible to test because reference specimens were not collected at the time. *Lindavia intermedia* was reported by Reid (2005) from Lakes Aviemore and Hayes, but whether lake snow was present at the time is uncertain. Of all the lakes from which the species is currently known in New Zealand, the latter is the only one that is significantly nutrient-enriched. Further information on *L. intermedia* is given in Novis et al. in press.

No historical record of this species in New Zealand has been found prior to the early 2000s. Furthermore, it is either absent or found only in surface layers of sediment cores from Lakes Wānaka, Wakatipu and Coleridge (É. Saulnier-Talbot, pers. comm., 2017; Novis unpublished observations), the only relevant lakes cored to date. This strongly suggests that the species is a recent invader of New Zealand. Testing this status is of obvious interest: if *L. intermedia* is not a recent arrival, the development of persistent lake snow is almost certainly due to a recent change in conditions in the Southern Lakes. If it is a recent adventive, different containment strategies should be considered and additional funding streams for research and remediation may be available.

Methods of DNA-based analysis can offer considerable additional information to test hypotheses of the origins of species, especially given the increasing affordability and speed offered by high-throughput DNA sequencing. Otago Regional Council (ORC) therefore contracted Landcare Research to address the origin of New Zealand *L. intermedia* using molecular methods.

2 Methods

2.1 Collection of New Zealand material for genome sequencing

Material was collected from Lakes Wānaka, Wakatipu, Hawea and Coleridge by dragging a weighted line from a boat trolling at low speed for 500−1,000 m. The Wānaka, Wakatipu and Hawea samples were provided by ORC. The material adhering to the line was scraped off and transported cold and fresh to the laboratory. Microscopic examination revealed all these samples to be well dominated (at least 95%) by *Lindavia intermedia.*

2.2 DNA extraction for genome sequencing

The samples were subjected to a protocol, established earlier, that successfully extracts DNA from fresh samples of lake snow (Novis et al. in press). Briefly, a large spoonful of material was resuspended in ADB buffer (Zymo Research, Irvine, CA, USA), which is usually intended to dissolve agarose gels, in 50 mL centrifuge tubes and incubated at 50–60°C for up to 2 h. The samples were then centrifuged briefly and resuspended in deionised water, before grinding and incubation in CTAB buffer for 30 min at 65°C. The material was centrifuged and the supernatant subjected to a phenol-chloroform extraction followed by clean-up in a Zymo Clean & Concentrate column (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. The resulting extracts were assessed using Nanodrop measurements, with the column step ultimately undertaken twice to satisfy DNA quality requirements for Illumina library preparation.

2.3 Shotgun genome sequencing and assembly

Paired-end 2 × 250 Illumina Miseq was carried out by New Zealand Genomics Ltd (NZGL), with all three samples pooled on a single lane. Sequence contigs were generated using the 'de novo assembly' option on the processed sequences obtained from NZGL in Geneious 8.1 (http://www.geneious.com; Kearse et al. 2012), and consensus sequences generated on the basis of at least 100× coverage, with sequences of <100 bp removed (leaving approximately 6 million reads for each of the three lake samples). Additional assemblies were run to obtain chloroplast genomes using the 'assemble to reference' option, with the published chloroplast genomes of *Cyclotella* sp. WC03_2 (Genbank accession number KJ958481) and *Cyclotella* sp. L04_2 (Genbank accession number KJ958480) as reference sequences.

2.4 Marker design

The following approaches were taken to marker design.

a. Chloroplast markers.

The assembled chloroplast genomes from the three lakes were aligned using the Geneious alignment function. Primer pairs were designed in regions where sequence diversity was suspected by comparison to the Cyclotella genomes referenced above. At least one of each primer pair was overlapped from a coding region into an intergenic spacer (conferring high specificity that would exclusively amplify the target from a mixed sample).

b. Manually designed nuclear markers.

Contigs from all three lakes were compared by eye to select repeat regions that appeared polymorphic. Primer pairs were then designed using sequences of high complexity bordering these repeat regions.

c. Automated design of nuclear markers.

The software MSATCOMMANDER v0.8.2 (Faircloth 2008) was used to generate primer pairs from the processed Wakatipu Miseq reads. The library was searched for di- to pentanucleotide repeat regions with at least four repeat units, and flanked by appropriate regions for primer design. Primers were designed via the default settings of Primer3 (Rozen & Skaletsky 2000) as implemented in MSATCOMMANDER. Fifty primer pairs were chosen for screening from the MSATCOMMANDER output to supplement the additional 42 primer pairs (25 chloroplast and 17 nuclear, above) designed from existing sequence information, using an M13 tag (TGTAAAACGACGGCCAGT) on the 5' end of the forward primer for subsequent fluorescent labelling. The 50 automated pairs were arbitrarily chosen from a range of expected fragment sizes.

2.5 Initial screening

Sixty-seven of the primer pairs were tested on two samples (from Lake Coleridge and Lake Wanaka). DNA was extracted by the standard CTAB method (Doyle & Dickson 1987), with modifications as above. PCR was performed in 15 µL reactions consisting of 1 µL DNA at 5−50 ng and final concentrations of 1 × KAPA Plant PCR Buffer + dNTPs (KAPABIOSYSTEMS, Cape Town, South Africa) (0.2 mM each dNTP and 1.5 mM $MgCl_2$), 0.3 U KAPA3G Plant DNA Polymerase (KAPABIOSYSTEMS), 0.08 µM forward M13 tagged primer, 0.32 µM reverse primer, and 0.32 µM M13 labelled primer. PCR conditions were as follows: 95°C 5 min; 30 cycles of 95°C 20 s, 57°C 15 s, 72°C 30 s; 15 cycles of 95°C 20 s, 51°C 15 s, 72°C 30 s; 72°C 10 min.

PCR products were separated on 2% agarose gels, and the 15 primers that produced clear bands for both samples and that were potentially polymorphic were then used to amplify additional samples (see below).

2.6 Obtaining foreign material

The following methods were undertaken to attempt to obtain samples containing *L. intermedia*.

- An initial request for material corresponding to the *Lindavia bodanica* complex was sent on the global email server Diatom-L (subscribers to which are generally practising diatomists). I received one response to this email, from Dr Will Daniels (Brown University), with whom I was already in contact, offering material from Alaskan lakes. Unfortunately the species proved to be absent from these samples.
- Drs Andrew Alverson (University of Arkansas) and Edward Theriot (University of Texas) were approached for DNA from their former cultured strain of *Cyclotella bodanica* (now *Lindavia bodanica*, by far the closest relative of *L. intermedia* for which published sequence data are currently available). This they agreed to provide, but unfortunately it could not be found in their freezer storage. Dr Alverson was able to send scanning electron micrographs of the strain (some of which are reproduced with permission in Novis et al. in press).
- Dr Moya Joubert (Seattle Public Utilities), with whom we were previously in contact, provided a sample rich in *L. intermedia* from Lake Youngs (Washington, USA), the only other site worldwide where the species is known to produce lake snow.
- Joanna Gauthier (McGill University) provided samples from Cultus Lake (British Columbia, Canada). These comprised a sediment sample and several DNA samples from the lake water column. The sediment was rich in *L. intermedia*, and one of the DNA samples was collected when *L. intermedia* was present. Contact with Joanna was established by our colleague Dr Émilie Saulnier-Talbot (affiliated with Laval University) during the 2016 International Diatom Symposium in Québec City.
- Dr Sergei Genkal, who has published on *L. intermedia* from Russia (Genkal et al. 2013), was approached through email and ResearchGate messaging. No response was received.
- Dr Peter Kroth (Universität Konstanz) was approached via a previous contact in Germany and agreed to arrange for samples to be collected and sent from Bodensee (Lake Constance; Germany, Switzerland and Austria). However, after communicating with a local phycologist it transpired that the species had not been found in Lake Constance for 5 years, so these samples were not collected.
- Dr Joachim Hürlimann (AquaPlus, Switzerland) was contacted directly to obtain samples from Zugersee (Lake Zug, Switzerland). However, unlike many European lakes it transpired that this lake is still eutrophic following enrichment in the mid-20th century. Consequently *Lindavia bodanicoid* species have not been recorded there since the 1950s, and no samples were collected.

- Dr Arielle Cordonier (DETA, Switzerland) was contacted via Dr Hürlimann and agreed to arrange collection of samples from Lac Léman (Lake Geneva; Switzerland, Italy and France). Her colleague, Sophie Lavigne, sampled the lake monthly between January and May 2017 with plankton tows and filtration. The sample collected in February was found to contain small numbers of *Lindavia*; the genus appeared to be absent from the other samples.

Table 1 lists the final samples and associated details that were successfully used in this project, and Figure 1 shows their locations in map form.

Table 1 Details of *Lindavia intermedia* samples from New Zealand and overseas used in this study

Site	Country	Approx. locality	Collector	Date	Allan Herbarium voucher
Lake Coleridge	New Zealand	43.333924S, 171.541807E	P. Novis, Landcare Research	22/7/16	CHR589916
Lake Hawea	New Zealand	44.590408S, 169.273291E	N. Manning, Otago Regional Council	26/6/17	-
Lake Wānaka	New Zealand	44.659884S, 169.100325E	A. Uytendaal, Otago Regional Council	23/9/16	CHR589915
Lake Wakatipu	New Zealand	45.056293S, 168.644162E	A. Uytendaal, Otago Regional Council	23/9/16	CHR589911
Lake Youngs	USA	47.419110N, 122.122227W	E. Johnson, Seattle Public Utilities	4/5/2017	CHR644492
Cultus Lake	Canada	49.050800N, 121.988273W	J. Gauthier, McGill University	?/6/15	CHR644493
Lac Léman (Lake Geneva)	Switzerland/ France/Italy	46.443638N, 6.481979E	S. Lavigne, DETA	21/2/17	CHR644494

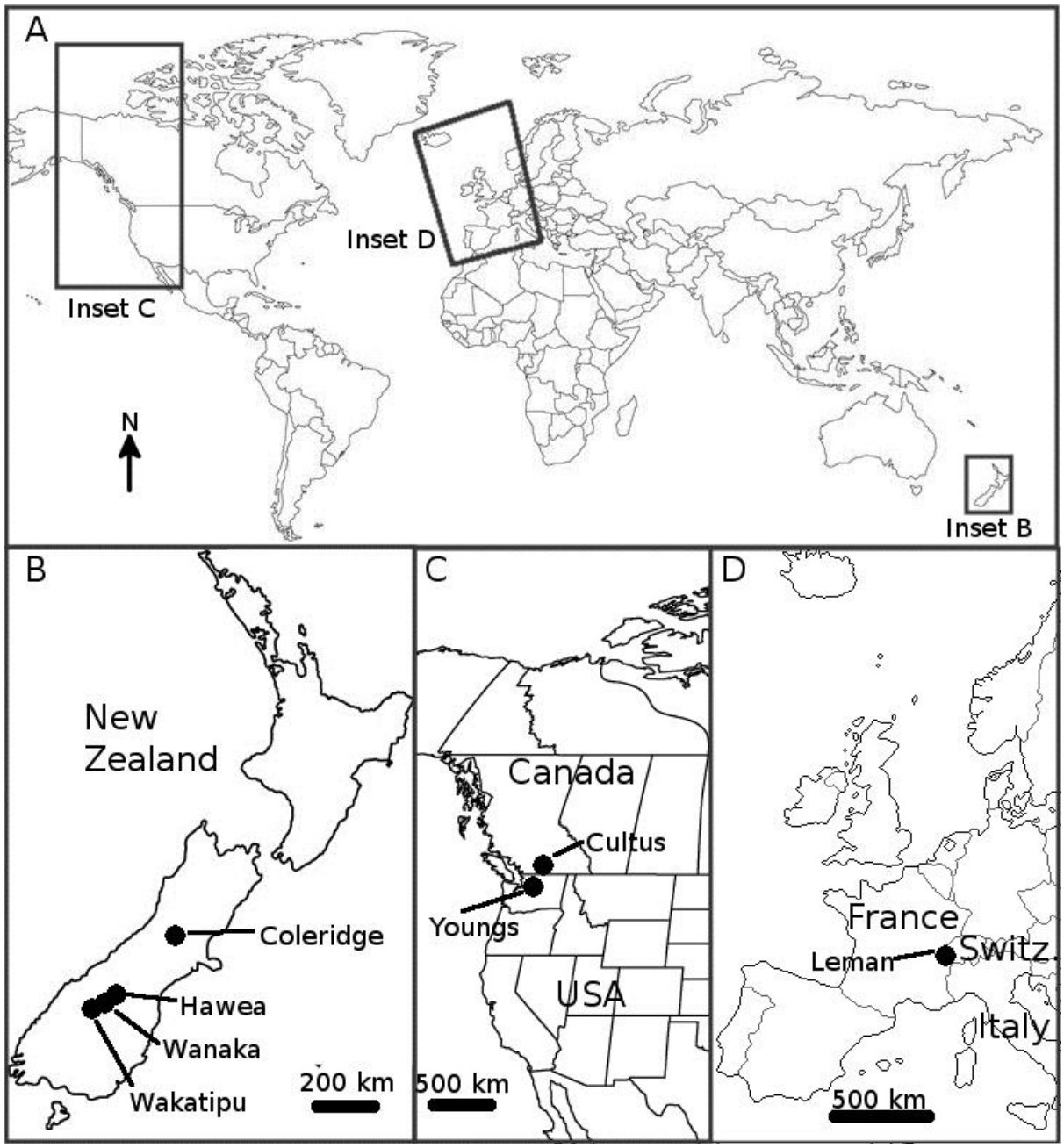

Figure 1 Maps showing the locations from which samples that were successfully sequenced using newly developed molecular markers were collected. A. World map showing relative locations of collecting areas. B. Collecting sites in New Zealand. C. Collecting sites in the Pacific Northwest of North America. D. Collecting site in Central Europe.

2.7 Morphological characterisation of new samples

Samples were examined on a Leica DMLB compound microscope with Nomarski Differential Interference Contrast optics, and images were taken using a Canon DS126271 digital camera. In order to observe valve ornamentation, diatoms were cleaned directly on coverslips using the muffle furnace method described by Biggs and Kilroy (2000), and permanent slides created using Naphrax (Brunel Microscopes Ltd, Wiltshire, UK) as a mountant. The diameters of valves and central areas and the number of striae per 10 µm (using the arc method) were measured on 40 valves from each sample for the Youngs and Cultus samples, and nine valves from the Léman sample (which contained much less material).

In order to observe ultrastructural surface features of diatoms in the samples, a cleaned coverslip from each sample was gold coated in a Desk Sputter Coater DSR1 (Nanostructured Coatings Co., Tehran, Iran) and examined on a TM3030Plus benchtop SEM (Hitachi, Tokyo, Japan). Typical operating conditions for the latter were 15 kV accelerating voltage and 5 mm working distance, using a mix of backscattered and secondary electron detectors, with the settings appropriate for conductive material.

2.8 Data acquisition

DNA from foreign samples was extracted either with the method described above or with a Maxwell 16 Tissue DNA purification kit (Promega Corporation, Madison, USA), or the DNA was received already extracted from a collaborator (in the case of Cultus Lake water column samples).

a. Nuclear markers.

The amplification method was as described above. One microlitre from each PCR product was added to 10 µL Hi-Di formamide (Applied Biosystems, Waltham, MA, USA) and 0.2 µL GeneScan 600 LIZ size standard (Applied Biosystems), before being separated on a 3500xl genetic analyser (Applied Biosystems) using a DS-33 dye set at the Landcare Research sequencing laboratory (Auckland, New Zealand). Fragments were visualised and scored using Geneious v8.0.5 (Biomatters, Auckland, New Zealand), and polymorphism at each locus was assessed. Seven of the loci tested produced polymorphic fragments and no more than two alleles per individual (Table 2). A further six amplified reliably but were monomorphic, and two produced results that were not scoreable. A genetic distance matrix and principal coordinates analysis (PCoA) was performed using GenAlEx v6.501, and a UPGMA tree was constructed in MVSP v3.22 (Kovach 2007) from the same genetic distance matrix. The markers were tested for repeatability by repeating a duplicate set, and three were retained as robust.

b. Chloroplast markers.

The 25 chloroplast primer pairs were used to sequence all samples, using the same PCR mix (omitting the M13 tagged primer and adjusting primer concentrations to equimolar) and the cycling conditions were as follows: 95°C 5 min; 35 cycles of 95°C 20 s, 57°C 15 s, 72°C 30 s; 72°C 10 min. Sequencing was performed on the same genetic analyser described above. Electropherograms were checked by eye and assembled using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). A multiple sequence alignment was created using MEGA6 (Tamura et al. 2013) from the four primer pairs that amplified products from all samples (except Lac Léman, which did not amplify reliably), data gaps and sites containing ambiguous nucleotides were omitted, and a UPGMA tree was created using MEGA6, with 'number of differences' as the distance metric (a more complex model was unnecessary, since all sequences were identical except one; see Results). Where possible, sequences from the Cultus Lake DNA sample extracted from water by Joanna Gauthier were compared to sequences from the Cultus Lake sample extracted from sediment (which were extracted by us and from which we could obtain morphological data); these were always identical.

The final set of six primer pairs is shown in Table 2.

Table 2 Characteristics of six polymorphic loci developed in *Lindavia intermedia*. The LN and SSR prefixes refer to nuclear markers, LC to chloroplast markers

Locus	Primer sequences (5'-3')[1]	Repeat motif[2]	Allele size range (bp)	T_a[3] (°C)
LN11	F: GCATATTTTATGTGCAGG R: TATGTTGTTCGATATACC	GA	436–454	57
LN26	F: TATGACAAAGGCCAGGGC R: CACTGCATCGGAAGGTGC	TA	194–196	57
SSR34	F: ATCACCACTAGCTACCGCTTG R: AACCGGACCAGCGATTTGG	CAG	141–151	57
LC6	F: AACTGAGTTCTAACAGCG R: GAAGAAAGCAAAATTGGC	-	195	57
LC17	F: CCCAGTAGGAATCGAACC R: AGCGGTAGAGTGTCTGCC	-	141	57
LC30	F: TAGCCTACGGAAGTAGCG R: GACCATTCGACATAATAG	-	120	57

[1]Note that an M13 tail (TGTAAAACGACGGCCAGT) was added to the 5' end of each forward primer.

[2]Not applicable for chloroplast markers, which were sequenced rather than scored by size, and distinguished taxa by base substitutions.

[3]Annealing temperature used in PCR.

3 Results

3.1 Morphological comparison of New Zealand and overseas specimens

A selection of scanning electron micrographs of *Lindavia intermedia* from New Zealand and overseas localities, recorded from the samples used in this study, is shown in Figure 2. Lake Youngs specimens were indistinguishable from New Zealand specimens according to stria density, cell size, and ratio of central area width to valve width (Figure 3). These characters are among those used to distinguish species within *Lindavia* (e.g. Novis et al. in press); Figure 2A indicates their positions on the diatom valve face.

However, Cultus Lake and Lac Léman specimens were slightly different, with a slightly increased range and median density of striae and central area:valve width ratio (Figure 3). Figure 4 places this in the context of other species of *Lindavia*: while the criteria placed the New Zealand and Lake Youngs material in agreement with currently accepted boundaries of the species *L. intermedia* (Daniels 2012), the Cultus Lake and Lac Léman specimens were found to range between these and the criteria currently accepted for *L. bodanica* (Burge & Edlund 2017).

3.2 Genetic comparison of New Zealand and overseas specimens

Each of the three nuclear markers and three chloroplast markers produced the same key result: all New Zealand material was identical to that from Lake Youngs. The sample from Cultus Lake differed from this slightly (in the case of the chloroplast markers, this difference amounted to seven base substitutions over 434 base pairs of chloroplast genome spacer region). The sample from Lac Léman amplified from only one of the three chloroplast markers; therefore, a conservative approach was taken and this sequences was excluded. If it was included, and considering that marker alone, the Lac Léman specimens were the most distant from the New Zealand specimens. The three robust nuclear markers amplified successfully from the Lac Léman sample, similarly placing it slightly more distant from the New Zealand material than the material from Cultus Lake. These results are shown in the form of a UPGMA phylogeny in Figure 5.

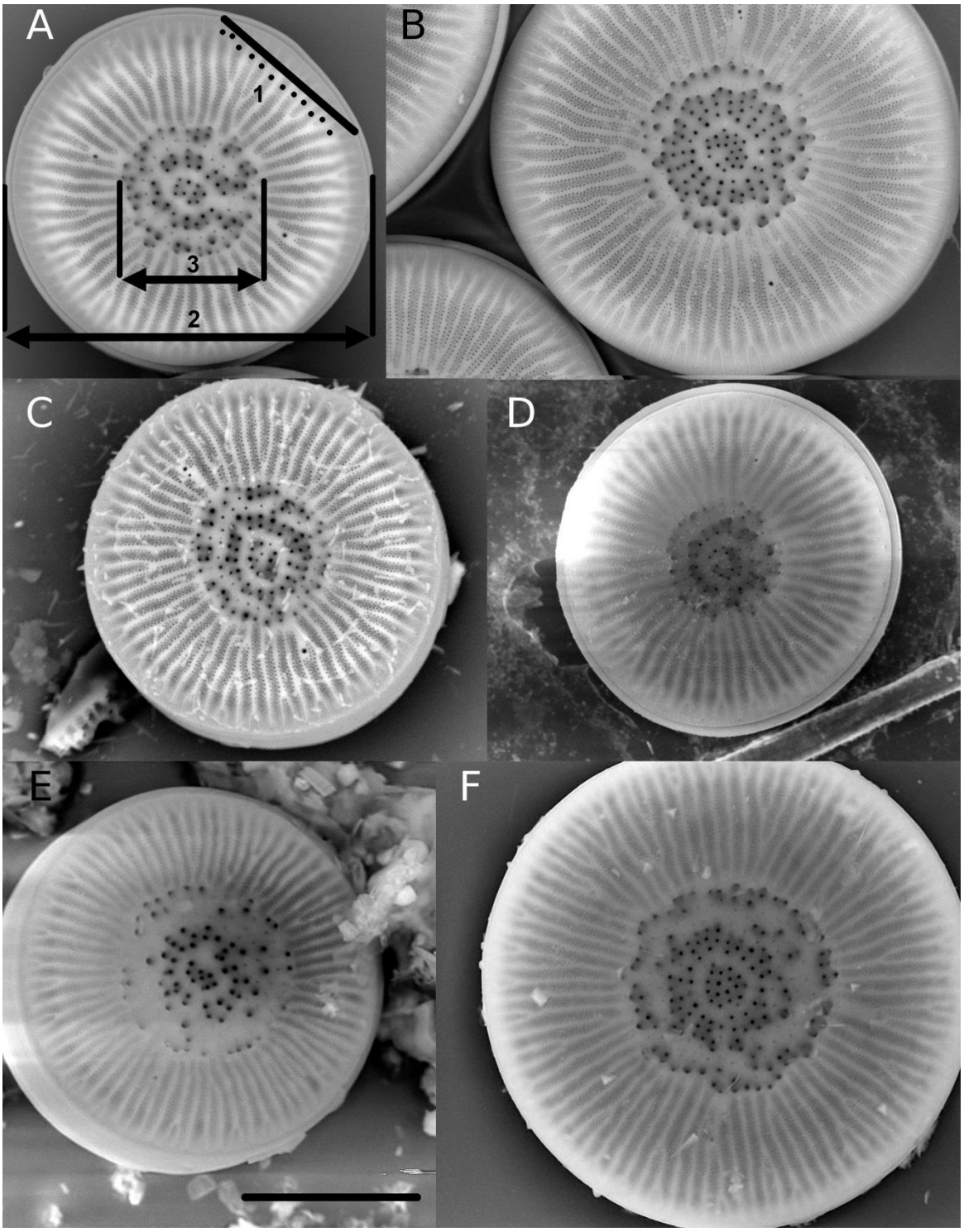

Figure 2 Scanning electron micrographs showing typical valves of *Lindavia intermedia* from New Zealand and elsewhere, and characters used in morphological analysis. A. Lake Coleridge, showing morphological characters enumerated in later figures: 1, number of striae (12 in this case) in a 10 µm arc; 2, cell width; 3, central area width, typically combined with cell width to give a ratio. B. Lake Wānaka. C. Lake Wakatipu. D. Lake Youngs (WA, USA). E. Cultus Lake (BC, Canada). F. Lac Léman (Switzerland). All shown at the same scale (the bar in E. is 10 µm).

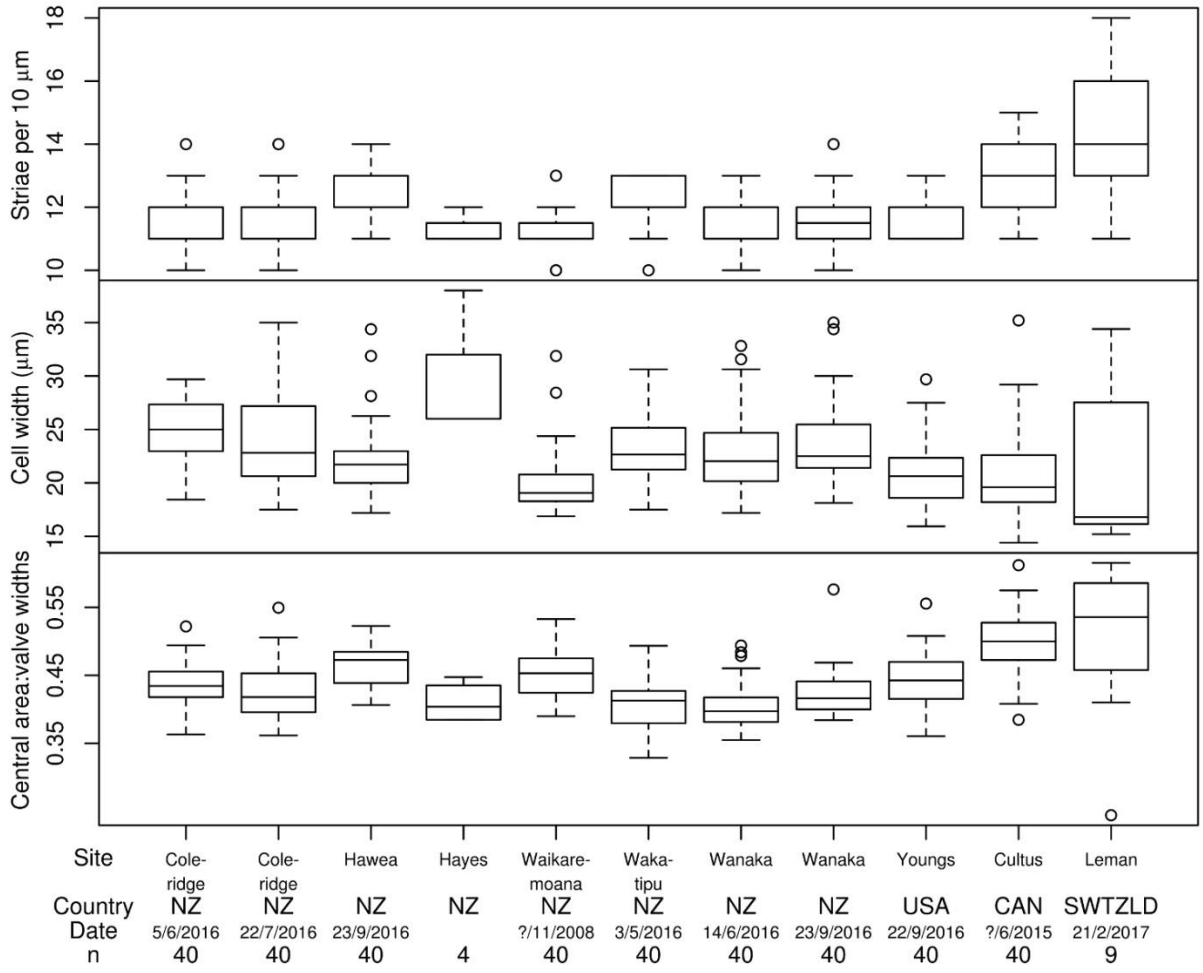

Figure 3 Values for three characters commonly used for identification of bodanicoid diatoms (see Figure 2A), scored for *Lindavia intermedia* populations in New Zealand and from populations from Lake Youngs (USA), Cultus Lake (Canada) and Lac Léman (Switzerland). Boxes define (from top to bottom) the upper quartile, median and lower quartiles; whiskers the limits of the nominal ranges inferred from the quartiles; and open circles the outliers. 'Date' refers to the date of collection, and 'n' to the number of individuals measured from the population.

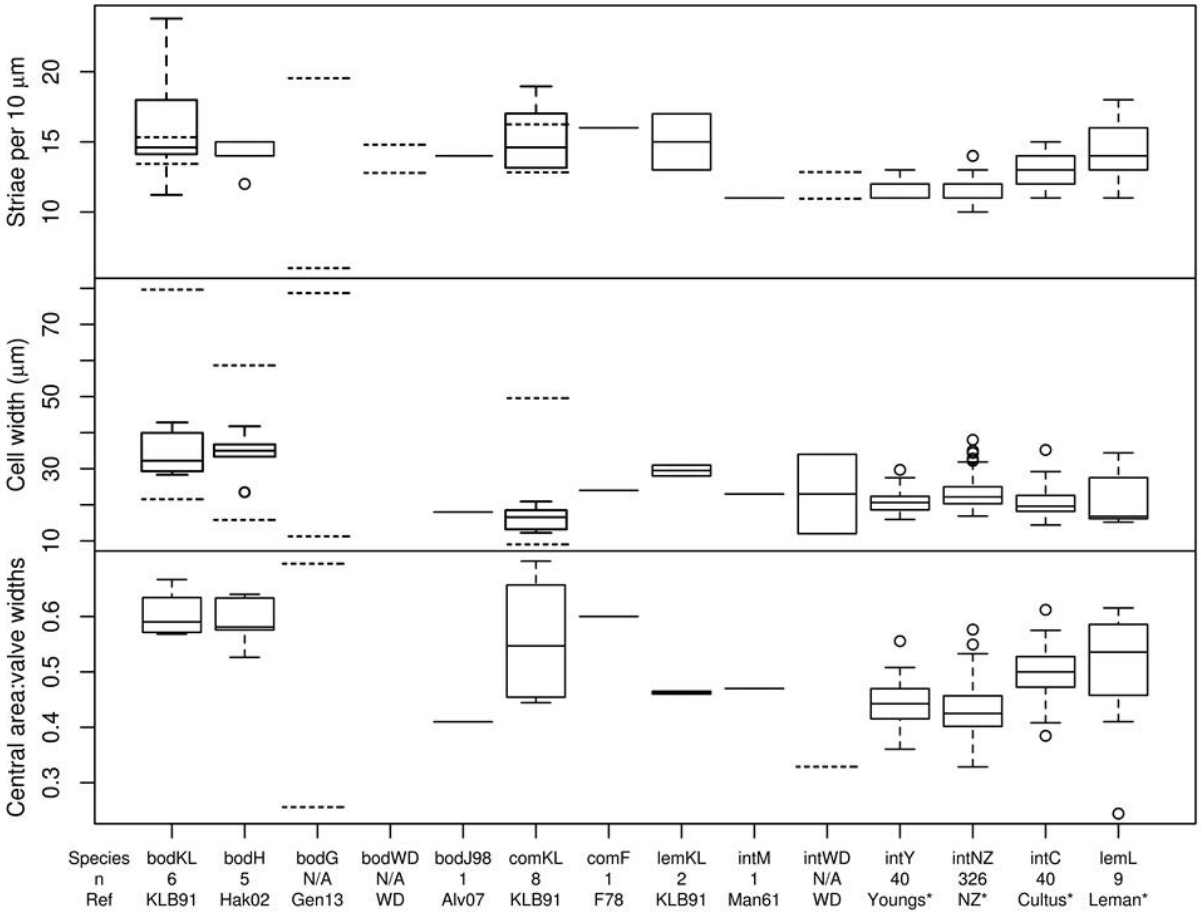

Figure 4 Values for the three characters scored in the previous figure, applied to representatives of the *Lindavia bodanica* complex from a variety of sources. Boxplots refer to measurements made on illustrative material from references or (in the case of Youngs, Cultus, Léman and New Zealand) on field populations. Dashed lines represent ranges specified in descriptions from the references given below. Under 'Species', 'bod' refers to *Lindavia bodanica*, 'com' to *L. comta*, 'int' to *L. intermedia*, and 'lem' to *L. lemanensis*. The initials following the species name refer to the associated reference or site. The identification of *L. lemanensis* for material from Lac Léman is assumed here but uncertain. References are: KLB91, Krammer & Lange-Bertalot 1991; Hak02, Häkansson 2002; Gen13, Genkal et al. 2013; WD, Western Diatoms; Alv07, Alverson et al. 2007; SEM images kindly sent by the author); F78, Foged 1978; Man61, Manguin 1961. Western Diatoms includes two references: Daniels 2012 for *L. intermedia* and the striae count for *L. bodanica*, and Burge & Edlund 2017 for the remaining data for *L. bodanica*. Asterisks refer to the populations measured by us from field material.

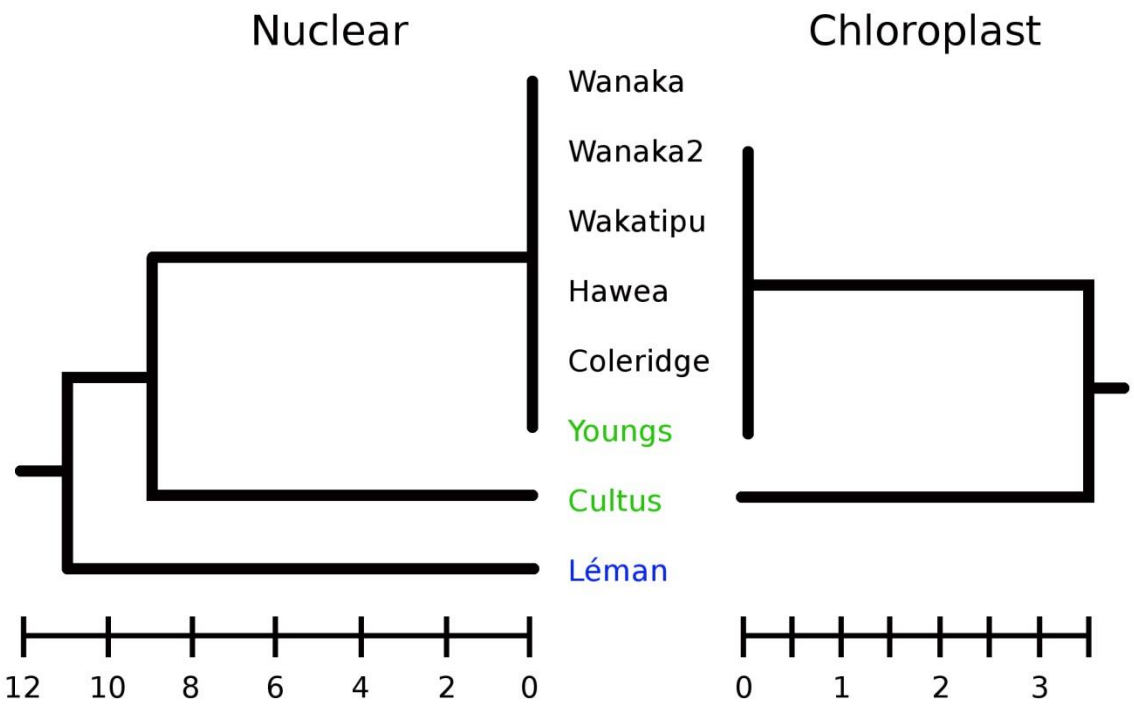

Figure 5 Trees constructed using the UPGMA method for genetic distance data generated from three newly developed nuclear (left) and chloroplast (right) molecular markers for *Lindavia intermedia*. New Zealand sites are in black, North American in green and European in blue. Scales refer to the codominant genetic distance for microsatellites calculated by GenAlEx for nuclear markers, and number of base substitutions for the chloroplast markers.

4 Discussion

Although tempered by the modest samples from overseas, this study produced a clear result. According to analysis of newly developed markers suitable for examining intraspecific variation, *Lindavia intermedia* from Lake Youngs (USA) is identical to the species currently producing lake snow in large, clean lakes in New Zealand, and different from other forms identified as *L. intermedia* and *L. lemanensis* (two species that Krammer and Lange-Bertalot (1991) placed in synonymy). Furthermore, morphological measurements tend to support the genetic data and indicate a range of values spanning the intervals currently circumscribing separate species (Daniels 2012; Burge & Edlund 2017). This is similar to findings published by Genkal et al. (2013), prompting them to propose synonymy of *L. intermedia* with *L. bodanica*. As described in Daniels et al. (2016) and Novis et al. (in press), the taxonomy of this group has a complicated history and species boundaries remain uncertain.

The original goal of the study was to determine whether *L. intermedia* is a recent invader to New Zealand and where it may have come from. The paucity of material we were able to obtain from overseas was undoubtedly a hindrance in addressing these questions. Nonetheless, some very pertinent information has been obtained.

- New Zealand *Lindavia intermedia* is genetically and morphologically identical to specimens from Lake Youngs (USA), which is the only other site worldwide in which this species conclusively produces significant quantities of lake snow. In Cultus Lake (Canada) it is not certain if lake snow occurs. There are apparently no reports of this phenomenon from fishers, but some researchers experience problems with net clogging at some times of the year, and this may not be during fishing season (J. Gauthier, McGill University, pers. comm., 2017).
- Specimens from Cultus Lake, which closely resemble the material from New Zealand and Lake Youngs and have been identified as *L. intermedia* by researchers working on the lake, belong to a different genotype. They also appear to differ slightly in morphology. There is thus a greater genotypic diversity within this species, as well as within the genus, in the Northern Hemisphere than occurs in New Zealand, where only one genotype (and species of *Lindavia* for that matter) appears to be present.
- Material corresponding to this species (or the possibly conspecific *L. lemanensis* or even *L. bodanica*) appears to have become scarce in the lakes of Europe. This is at least partly due to the trophic perturbations experienced by these lakes over the previous 60 years. In some cases, such as Zugersee, eutrophication persists and excludes *Lindavia*; the genus has recolonised other lakes following phosphorous reduction programmes and oligotrophication. However, the populations persisting in European lakes prior to eutrophication in the mid-20th century were doubtless exterminated by the enrichment (as shown in the sedimentary record of Zugersee; Elber et al. 2001). Whether the restored populations reflect the earlier genotypes is unknown, and probably impossible to test. This is unfortunate, since some of these lakes were type localities for species of *Lindavia* (*L. bodanica* from Bodensee, *L. lemanensis* from Lac Léman). The increased rarity of *Lindavia* in these lakes also reduces the chance of Europe being a source of new material in New Zealand, consistent with the result in Figure 5.
- Sediment core samples taken from Lakes Wanaka, Wakatipu and Coleridge all indicate that the species has appeared in these lakes in the recent past (M. Schallenberg, University of Otago, and É. Saulnier-Talbot, pers. comms., 2017).

Taken together, these observations represent a strong circumstantial case for transfer of *Lindavia intermedia* to New Zealand from the Northern Hemisphere, particularly North America.

Given the presence of the same genotype in Lake Youngs as in New Zealand, it may be worth reviewing knowledge of the environment of Lake Youngs. It is a water reservoir for local communities and is therefore relatively clean. Published data on the lake are scarce; the best source found is Canale et al. 1997, which predates the occurrence of lake snow in Lake Youngs, but provides some indication of its water quality (phosphorous loadings are not thought to have risen prior to the advent of lake snow, unless possibly from the deep sediments; Zisette 2009). These data are compared with other lakes in the study in Table 3. The two North American lakes are notably smaller than the New Zealand lakes and Lac Léman, but are deep enough to stratify, and all three foreign lakes contain more phosphorous than the New Zealand lakes. However, none would be categorised as more than mesotrophic.

Table 3 Selected properties of the lakes in this study, based on the variables available for Lake Youngs in Canale et al. 1997

Lake	Maximum depth (m)	Surface area (km^2)	Total P ($\mu g\ L^{-1}$)	Source[1]
Coleridge	200	36.9	2–5	LAWA
Hawea	384	137.6	2–7	LAWA
Wānaka	311	180	2–7	LAWA
Wakatipu	380	289	2	LAWA
Youngs	30.5	2.8	4–15	Canale et al. 1997[2]
Cultus	41.8	6.3	4.3–15	Selbie 2013
Léman	309	580	12–20	Jacquet et al. 2014

[1] LAWA = Land, Air, Water Aotearoa. website: www.lawa.org.nz

[2] Reporting data from 1992.

It is tempting to hypothesise Lake Youngs as a possible source of *Lindavia* in New Zealand. Although Lake Youngs is a reservoir for local drinking water, and is nominally fenced off from the public, this probably does not isolate it from being a potential source of material for transfer elsewhere. Local internet forums can easily be found in which subscribers enthusiastically describe the size of the fish in Lake Youngs and recommend that others 'hop the fence ... and start casting' (e.g. www.washingtonflyfishing.com/forum/threads/why-no-trophy-lake-in-south-king-county.46625/; www.northwestfishingreports.com/forum/viewtopic.php?t=3812).

However, counter to the hypothesis of Lake Youngs as a possible source of New Zealand *Lindavia* is the timing of lake snow in that lake, first reported in earnest in 2008 (Zisette 2009). The phenomenon is thought to have begun prior to this in Lake Wānaka, with the species thought to have become detectable in lake sediments in approximately 2004 (É. Saulnier-Talbot, pers. comm., 2017). The earliest suspected lake snow event in New Zealand is in Lake Benmore in the early 2000s. However, it does not appear to be known how long *L. intermedia* has been present in Lake Youngs prior to the onset of lake snow production.

Another strong possibility is that other North American lakes harbour this species without lake snow being produced or perhaps detected; the latter could be quite likely in more isolated alpine lakes. Regardless, it now seems very likely that the genotype responsible for lake snow diversified somewhere in the Northern Hemisphere prior to a recent invasion of New Zealand, either natural or human-mediated.

5 Recommendations

A large number of pressing questions remain to be addressed. One of these, highlighted by the present study is: Why is lake snow produced by *L. intermedia* so rare in the rest of the world? A related question is, Why has it proven persistent in some lakes (Wānaka since at least 2008, Coleridge since 2015) but apparently ephemerally in others (probably in Benmore; confirmed in Waikaremoana)? The New Zealand lakes that have been cored to date are those in which lake snow has been persistent, and demonstrate a gradual increase in *L. intermedia* in the core surface layers; sediment cores taken from Benmore and Waikaremoana may reveal much more periodicity, which could help to link abundances to water chemistry and thus population drivers, where such data are available. Coring a wider variety of lakes is therefore an important line to pursue.

Determining possible management interventions requires more than cell quantification, however. Methods for accurately quantifying lake snow production must be developed to determine the extent of its decoupling from population growth and the conditions under which it occurs. If lake snow consists largely of β-chitin, as seems likely (Gügi et al. 2015), large-scale production of this nitrogen-containing polysaccharide may indicate a delicate imbalance between available nitrogen and phosphorous. Ultimately, laboratory experiments will be needed to study the physiology of *L. intermedia*, requiring the ability to culture the organism. A close relative from Wyoming was cultured with difficulty by Alverson et al. (2007); our newly established contact with these researchers would probably prove very useful in efforts to grow the New Zealand material artificially.

The effect of *Lindavia* on lake ecology also demands attention. Lake snow transforms the water column of microtrophic lakes from essentially a carbon ‘desert’ to a series of carbon- (and potentially nitrogen-) rich oases much more favourable for bacterial growth. The further impact of this on higher trophic levels in the food chain is unknown, but suggests a tipping-point scenario towards further change in species and resource composition of lake ecosystems.

The molecular markers developed here have the potential to unravel both the systematics and biogeography of bodanicoid *Lindavia*. This would resolve long-standing taxonomic problems in the group and greatly assist with the biosecurity implications arising from our results; i.e. that this species should be regarded as an adventive that may threaten more lakes worldwide, and thus needs to be accurately detectable. Given better samples, more markers would likely be available for use. Such an enterprise would best be undertaken in a more coordinated fashion, with more time and funding available (i.e. at a national level) than has been available for this study.

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Appendix 1 – Fragment sizes recorded in each sample for the three robust (repeatable) nuclear markers developed in this study.

Lake	Country	Nuclear marker fragment sizes		
		LN11	LN26	SSR43
Coleridge	New Zealand	441, 454	196	151
Hawea	New Zealand	441, 454	196	151
Wānaka	New Zealand	441, 454	196	151
Wānaka 2	New Zealand	441, 454	196	151
Wakatipu	New Zealand	441, 454	196	151
Youngs	USA	441, 454	196	151
Cultus	Canada	436, 454	194	145
Léman	Switzerland/France/Italy	392, 392	175	143

Appendix 2 – Concatenated sequences recorded in each sample for the three robust chloroplast markers developed in this study (marker order is LC6, LC17, LC30).

>Coleridge

ACTGAGTTCTAACAGCGAGCTTATTAATATGGAAAGGTTGATTTTATCAATTTTACAAATTAATAAAAAAT
TTTTAAATAAAATAACCTAAGTTAAACTTAGATTATTTTAACGTTTGAATAATTGTTTTTTAGCTGACTTT
AATTTTTTTAAATTACACAGTTTTTGATAGAGCAGCCAATTTTTCCCAGTAGGAATCGAACCTACATTAGA
AACTTAGAAGGTTCCTGTCCTGATCCGTTAGACGATAGGCCCAAACAAAAAGCAATGGGTAATACAGGATT
TGAACCTGTGACCTTCTGCTTGTAAGGCATTAGCCTACGGAAGTAGCGTTCTTTAAGAGAAAAACTAATAT
TTGGACATTAAAAAAGATTCTTTACAAACCTCAACCATATGTAAAAACTATTAAGGAAACTCTATTATGTC
GAATGGTC

>Youngs

ACTGAGTTCTAACAGCGAGCTTATTAATATGGAAAGGTTGATTTTATCAATTTTACAAATTAATAAAAAAT
TTTTAAATAAAATAACCTAAGTTAAACTTAGATTATTTTAACGTTTGAATAATTGTTTTTTAGCTGACTTT
AATTTTTTTAAATTACACAGTTTTTGATAGAGCAGCCAATTTTTCCCAGTAGGAATCGARCCTACATTAGA
AACTTAGAAGGTTCCTGTCCTGATCCGTTAGACGATAGGCCCAAACAAAAAGCAATGGGTAATACAGGATT
TGAACCTGTGACCTTCTGCTTGTAAGGCATTAGCCTACGGAAGTAGCGTTCTTTAAGAGAAAAACTAATAT
TTGGACATTAAAAAAGATTCTTTACAAACCTCAACCATATGTAAAAACTATTAAGGAAACTCTATTATGTC
GAATGGTC

>Wanaka

ACTGAGTTCTAACAGCGAGCTTATTAATATGGAAAGGTTGATTTTATCAATTTTACAAATTAATAAAAAAT
TTTTAAATAAAATAACCTAAGTTAAACTTAGATTATTTTAACGTTTGAATAATTGTTTTTTAGCTGACTTT
AATTTTTTTAAATTACACAGTTTTTGATAGAGCAGCCAATTTTTCCCAGTAGGAATCGAACCTACATTAGA
AACTTAGAAGGTTCCTGTCCTGATCCGTTAGACGATAGGCCCAAACAAAAAGCAATGGGTAATACAGGATT
TGAACCTGTGACCTTCTGCTTGTAAGGCATTAGCCTACGGAAGTAGCGTTCTTTAAGAGAAAAACTAATAT
TTGGACATTAAAAAAGATTCTTTACAAACCTCAACCATATGTAAAAACTATTAAGGAAACTCTATTATGTC
GAATGGTC

>Hawea

ACTGAGTTCTAACAGCGAGCTTATTAATATGGAAAGGTTGATTTTATCAATTTTACAAATTAATAAAAAAT
TTTTAAATAAAATAACCTAAGTTAAACTTAGATTATTTTAACGTTTGAATAATTGTTTTTTAGCTGACTTT
AATTTTTTTAAATTACACAGTTTTTGATAGAGCAGCCAATTTTTCCCAGTAGGAATCGAACCTACATTAGA
AACTTAGAAGGTTCCTGTCCTGATCCGTTAGACGATAGGCCCAAACAAAAAGCAATGGGTAATACAGGATT
TGAACCTGTGACCTTCTGCTTGTAAGGCATTAGCCTACGGAAGTAGCGTTCTTTAAGAGAAAAACTAATAT
TTGGACATTAAAAAAGATTCTTTACAAACCTCAACCATATGTAAAAACTATTAAGGAAACTCTATTATGTC
GAATGGTC

>Wakatipu

ACTGAGTTCTAACAGCGAGCTTATTAATATGGAAAGGTTGATTTTATCAATTTTACAAATTAATAAAAAAT
TTTTAAATAAAATAACCTAAGTTAAACTTAGATTATTTTAACGTTTGAATAATTGTTTTTTAGCTGACTTT
AATTTTTTTAAATTACACAGTTTTTGATAGAGCAGCCAATTTTTCCCAGTAGGAATCGAACCTACATTAGA
AACTTAGAAGGTTCCTGTCCTGATCCGTTAGACGATAGGCCCAAACAAAAAGCAATGGGTAATACAGGATT

TGAACCTGTGACCTTCTGCTTGTAAGGCATTAGCCTACGGAAGTAGCGTTCTTTAAGAGAAAAACTAATAT
TTGGACATTAAAAAAGATTCTTTACAAACCTCAACCATATGTAAAAACTATTAAGGAAACTCTATTATGTC
GAATGGTC

>Cultus

ACTGAGTTCTAACAGCGAGCTTATTAATATGGAAAGGTTGATTTTATTAATTTTACAAATTAATAAAAAAT
TTTTAAATAAAATAACCTAAGTTAAACTTAGATTATTTTAACGTTTGAATAACTGTTTTTTAGCTGACTTT
AATTTTTTTGAATCACACAGTTTTTGATAGAGCAGCCAATTTTTCCCAGTAGGAATCGAACCTACATTAGA
AACTTAGAAGGTTCCTGTCCTGATCCGTTAGACGATAGGCCCAAATAAAAAGCAATGGGTAATACAGGATT
TGAACCTGTGACCTTCTGCTTGTAAGGCATTAGCCTACGGAAGTAGCGTTCTTCAAGAGAAAAACTAATAT
TTSGACATTAAAAAAGATTCTTTACGAACCTCAACCATATGTAAAAACTATTAAGGAAACTCTATTATGTC
GAATGGTC