

Molecular and Morphological investigations of *Pseudo-nitzschia* species isolated from Irish Waters.

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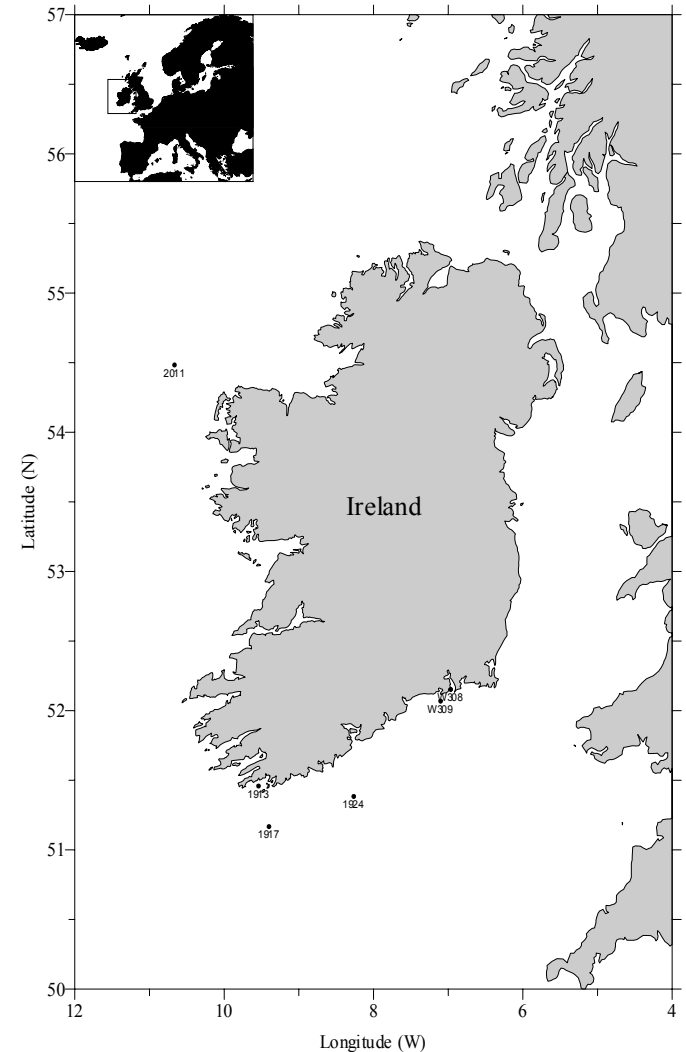
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Cultured isolates

SPECIES	REF.	LOCAL DESCRIPTION
<i>P. delicatissima</i> * ⁺	1913	Long Island Bay, SW coast
<i>P. delicatissima</i> * ⁺	1917	Off Fastnet rock, SW coast
<i>P. delicatissima</i> ⁺	1924-3	Shelf position off Cork, S coast
<i>P. delicatissima</i> ⁺	1424-4	Shelf position off Cork, S coast
<i>P. fraudulenta</i> * ⁺	W ₂	Waterford Harbour, S coast
<i>P. fraudulenta</i> ⁺	2011	Off Erris Head, Sligo, W coast
<i>P. pungens</i> * ⁺	WW ₃	Waterford Harbour, S coast
<i>P. australis</i> ⁺	WW ₄	Waterford Harbour, S coast

**Pseudo-nitzschia* isolates sequenced,

⁺*Pseudo-nitzschia* isolates screened with rDNA probes

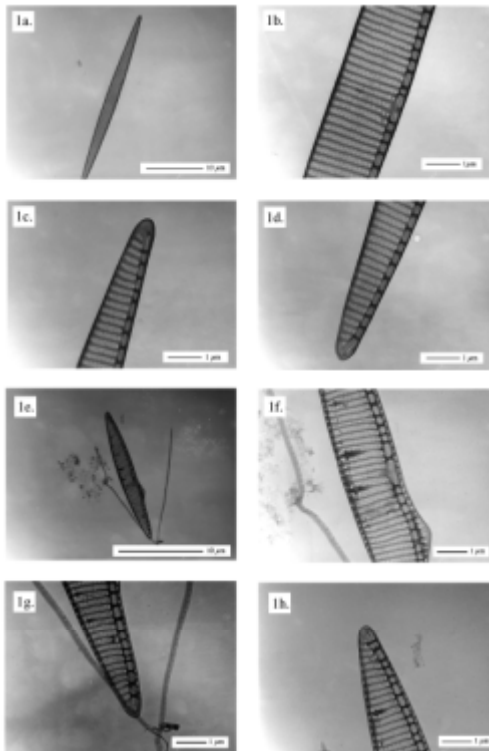


Morphology of cultured isolates

Transmission electron micrographs of *Pseudo-nitzschia* species selected for molecular analysis.

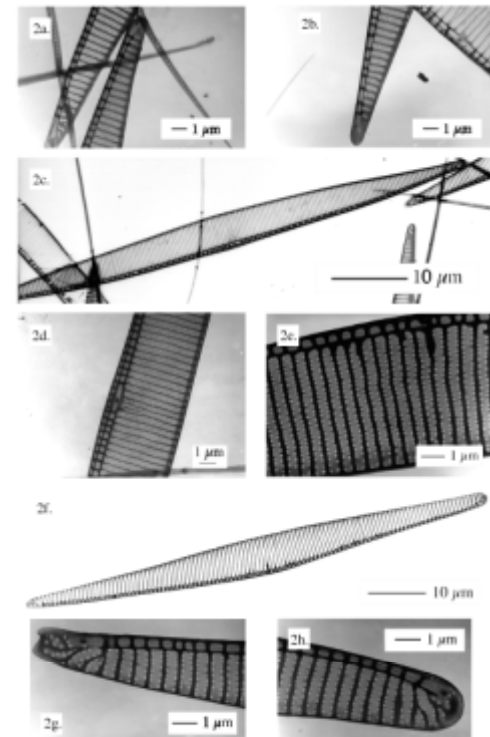
1a –1d *P. delicatissima* (1917)

1e –1h *P. delicatissima* (1913)



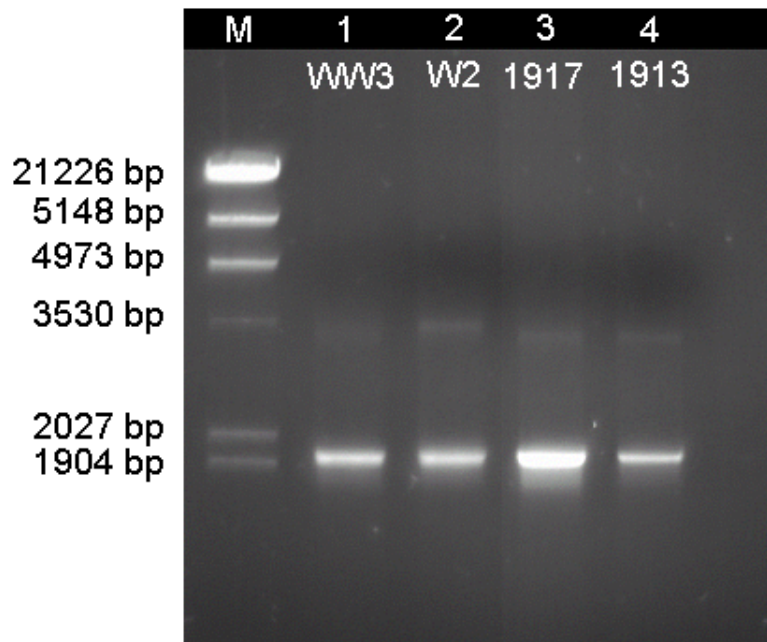
2a –2d *P. fraudulenta* (W₂)

2e –2h *P. australis* (WW₄).



Molecular techniques

DNA extraction, PCR amplification, TA cloning & Sequencing



- SSU
 - *P. delicatissima* 1745 bp (1913 & 1917)
 - *P. fraudulenta* 1745 bp (W2)
 - *P. pungens* 1743 bp (WW3)
- ITS1:
 - *P. delicatissima* 249 bp (1913 & 1917)
 - *P. fraudulenta* 286 bp (W2)
 - *P. pungens* 260 bp (WW3)
- 5.8S fragment (43-44 bp)

A sharp band of ~ 2,000 bp in length was amplified by PCR from genomic DNA of the 4 *Pseudo-nitzschia* isolates

Pairwise distances of clones

Similarity calculated using uncorrected-p analysis of the 18S SSU rDNA.

Taxa	1	2	3	4	5	6	7	8	9	10	11
1	-										
2	0.9971	-									
3	0.9885	0.988	-								
4	0.984	0.9834	0.984	-							
5	0.9891	0.9885	0.988	0.9885	-						
6	0.9874	0.9868	0.9862	0.9868	0.9983	-					
7	0.9874	0.9868	0.9862	0.9868	0.9983	0.9966	-				
8	0.9874	0.9868	0.9862	0.9868	0.9983	0.9966	0.9977	-			
9	0.9822	0.9816	0.9811	0.9834	0.9885	0.9868	0.9868	0.9868	-		
10	0.9868	0.9862	0.9857	0.988	0.9926	0.9908	0.9908	0.9908	0.996	-	
11	0.9868	0.9863	0.9845	0.9868	0.992	0.9903	0.9903	0.9903	0.9954	0.9994	-

High identity between characters within the genus of 98.12- 99.94%

P. delicatissima strains (99.71%),

P. multiseriis (99.66-99.83%)

P. pungens (99.54-99.94%).

1. *P. delicatissima* (1917)

4. *P. australis* (PSEUD-X)

7. *P. multiseriis* (NPARL)

10. *P. pungens* (BRUDC-X)

2. *P. delicatissima* (1913)

5. *P. multiseriis* (TKA-2)

8. *P. multiseriis* (POM-X)

11. *P. pungens* (F310).

3. *P. fraudulenta* (W2)

6. *P. multiseriis* (13CC)

9. *P. pungens* (WW3)

SSU 18S rDNA phylogenetic analysis

- Phylogenetic tree of SSU rDNA sequences
 - 1732 unambiguous characters used
- Constructed using Kimura 2 distances
 - The oomycete water mould, *Achlya bisexualis* (#M32705) was used as the outgroup
- Numbers at the nodes are bootstrap values for 100 bootstrap resamplings
 - Distance (above line)
 - parsimony (below the line)
 - tree length = 892

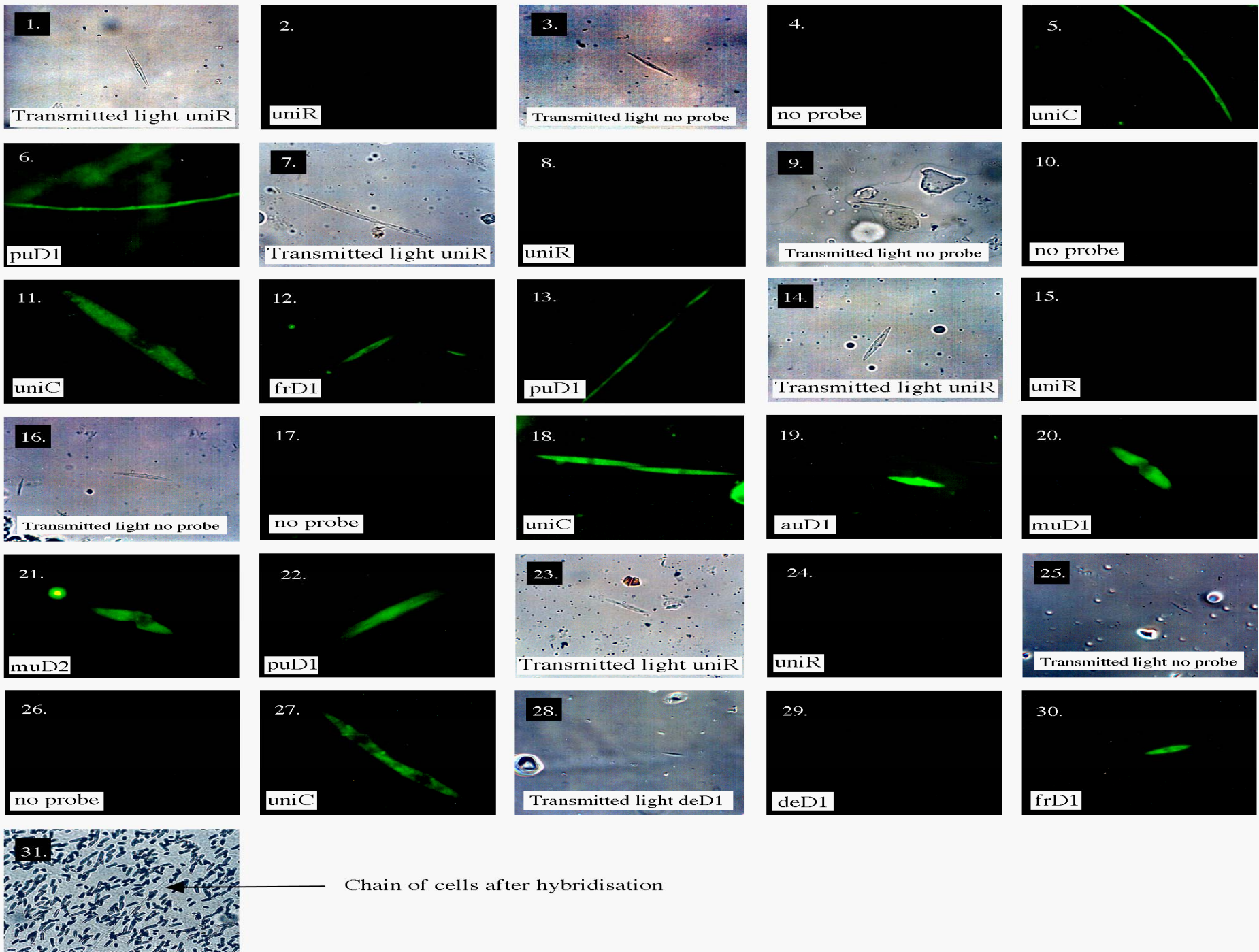
Application of LSU rRNA-targeted fluorescently labelled oligonucleotide probes

LSU rRNA-targeted oligonucleotide probes and their target species (Miller and Scholin 1996).

Probe	Target species
uniC	positive control (all organisms).
uniR	negative control.
muD1	<i>Pseudo-nitzschia multiseri</i> es (++).
muD2	<i>P. multiseri</i> es (++) , <i>P. pseudodelicatissima</i> (++) .
puD1	<i>P. pungens</i> (++) .
auD1	<i>P. australis</i> (++) .
frD1	<i>P. fraudulenta</i> (++) , <i>P. delicatissima</i> (+-) , <i>P. heimii</i> (+-) .
deD1	<i>P. delicatissima</i> (++) .
heD2-2	<i>P. heimii</i> (++) .
amD1	<i>Nitzschia americana</i> (++) . <i>N. americana</i> is no longer considered to be part of the genus <i>Pseudo-nitzschia</i> (Hasle and Syversten 1997)

Application of LSU rRNA-targeted fluorescently labelled oligonucleotide probes

Species	strain	Fluorescent oligonucleotide probe										
		uniC	uniR	No probe	miD1	miD2	puD1	auD1	fiD1	deD1	he2-2	amD1
<i>P. delicatissima</i>	1913	++	-	-	+-	-	-	+-	++	-	-	-
<i>P. delicatissima</i>	1917	++	-	-	+-	-	-	-	++	-	-	-
<i>P. delicatissima</i>	1924-3	++	-	-	-	-	-	-	++	-	-	-
<i>P. delicatissima</i>	19244	++	-	-	-	-	-	-	++	-	-	-
<i>P. pungens</i>	WW3	++	-	-	-	-	++/+-	-	-	+-	-	-
<i>P. australis</i>	WW4	++	-	-	+-	+-	+-	++	-	+-	-	-
<i>P. fraudulenta</i>	2011	++	-	-	-	-	+-	-	++	-	-	-
<i>P. fraudulenta</i>	W2	++	-	-	-	-	+-	-	++	-	-	-



Discussion and future work

- Morphology vs. Molecular analysis
- LSU rDNA probes: cross reactivity observed
- Future work: HEA project to develop probes for Irish *Pseudo-nitzschia* strains.