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5

6 **Reproductive strategies of *Lessonia berteroana***
7 **(Laminariales, Phaeophyceae) gametophytes from Chile:**
8 **Apogamy, parthenogenesis and cross-fertility with *L.***
9 ***spicata***

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11

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32 **Abstract**

33

34 *Lessonia berteroana* is one of the most exploited seaweeds in the Southeastern Pacific,
35 and its populations are recurrently facing overexploitation in northern Chile. Since
36 germplasms are not available, we decided to start gametophyte biobanking to support
37 conservation measures for this important resource in the future. Spores of *L. berteroana*
38 from nine localities at the Atacama coast were used to establish clonal male and female
39 gametophyte cultures. Unexpectedly, after isolation and under low light conditions,
40 juvenile sporophytes originated from somatic cells via apogamy, in most of female
41 gametophyte strains. In addition, eggs from solitary female gametophytes from Caleta
42 Cisnes and Torres del Inca had a strong tendency to generate sporophytes by
43 parthenogenesis, some of them even under low light regimes. Contrarily, female
44 gametophytes of the sister species *L. spicata* from southern Chile showed no evidence
45 for apomixis. When one of these *L. berteroana* strains is cross-fertilized with *L. spicata*,
46 true hybrids emerged based on the presence of eggs and subsequent sperm attraction.
47 These observations contrast with i) kelp recruitment assumed to be majorly by sexual
48 reproduction and ii) the strict reproductive separation of the two taxa reported for natural
49 populations within their contact zone at 30°S, and highlight their consideration for future
50 repopulation and breeding programs of *Lessonia*.

51

52

53

54 **Keywords:**

55 *Lessonia berteroana*, *L. spicata*, apogamy, apomixis, interspecific hybridization, kelp, life
56 history, parthenogenesis

57

58 Introduction

59

60 The brown algal genus *Lessonia* grows in large kelp beds along the coast of Chile. Some
61 of them represent natural resources of great economic and social importance. The
62 subtidal *Lessonia trabeculata* is predominantly used as forage for the mollusk abalone
63 (blades), which is a high-value export product, but its hard stipes are rich in low-M:G-
64 ratio alginates (Peteiro 2018). *Lessonia berteriana* and *L. spicata* occupy wave-exposed
65 intertidal stretches, and offer a high-quality alginate, but rich in high M:G ratio (Percival et
66 al. 1983). These two kelps are remarkably exploited seaweeds in Chile, and currently
67 subjected to severe over-exploitation. This has inspired numerous studies on natural kelp
68 beds, motivated by efforts to understand population dynamics as a base for the
69 development of management plans and harvesting strategies (summarized in
70 Westermeier et al. 2018). However, a serious deficit of fundamental data remains for
71 Chilean kelps: There is an almost complete lack of knowledge on their microscopic
72 stages, which are obligatory parts of their life cycles. The majority of published studies
73 deals with kelp beds on a macro-scale, taxonomy and molecular population genetics.
74 Consequently, life histories and sexual reproduction, including Mendelian genetics, are
75 un-explored, although knowledge in these fields is an indispensable basis for crop
76 improvement and future breeding programs.

77

78 Since no details beyond these general features were known for Chilean kelps, we have
79 started years ago to initiate pilot-scale clonal gametophyte cultures of *Lessonia*
80 *trabeculata* and *Macrocystis pyrifera* (Westermeier et al. 2006). We found that such
81 cultures can be maintained for many years and used as stock material for mariculture
82 projects. Subsequently, we showed, that heterosis breeding in *Macrocystis pyrifera* is
83 possible by mating parents of complementary genotypes (Westermeier et al. 2011), and
84 we also detected diploid male gametophytes in natural habitats (Müller et al. 2016).
85 Breeding programs for *Lessonia* species are lacking in Chile, although culture techniques
86 have been described to obtain small sporophytes in the laboratory (Hoffmann and
87 Santelices 1982).

88

89 Brown algal kelps are members of the order Laminariales and exhibit haplo-diploid life
90 histories: A haploid microscopic dioecious gametophyte generation produces eggs and
91 spermatozoids. Zygotes develop to the macroscopic diploid kelp sporophyte, which re-
92 establishes the haplophase via meiosis. Haploid meio-spores are formed in sori located
93 on reproductive parts of the thallus. Sometimes, however, gametophytes can undergo

94 deviations towards asexual reproduction (Oppliger et al. 2007). The term apomixis
95 stands for non-sexual origin of a sporophyte in haplo-diploid life histories. This deviation
96 from the standard life history pattern has been described for a plethora of terrestrial seed
97 plants (Bicknell and Koltunow 2004) and is also not uncommon in kelps (Nakahara and
98 Nakamura 1973; Le Gall et al. 1996; Oppliger et al. 2007). Two different mechanisms
99 may be distinguished: parthenogenesis, where an unfertilized egg produces a haploid,
100 genetically female sporophyte, and apogamy, where a sporophyte emerges directly from
101 a somatic cell of a gametophyte. Since apomixis products are often developed
102 abnormally and died in the early stages of life, their actual contribution to ecological
103 aspects is under-documented. However, sometimes parthenogenetic kelps may indeed
104 perform better than sporophytes derived from sexual reproduction, which suggested an
105 unexplored role in the field and a potential opportunity for mariculture

106

107 *Lessonia berteroana* and *L. spicata* are endemic for the southeastern Pacific. Due to
108 their habitats in the rocky exposed high-energy intertidal zone, mariculture attempts are
109 difficult. Up to the present harvest depends exclusively on natural stocks, endangering
110 the availability of the resource. In analogy to our previous strategies with *Macrocystis*, we
111 decided to evaluate the basis for a germplasm collection, in order to conserve seedstock
112 of both species, and to study their life history in detail. In these screenings we recurrently
113 found deviations of the life cycle towards apomixis, which we aim to describe in this
114 study.

115

116 **Materials and Methods**

117

118 Fertile sporophytes of *Lessonia spicata* were collected at Mar Brava, Chiloé in January
119 1997 and September 1999 (Fig. 1, Table S1). Gametophyte isolation and maintenance
120 proceeded as stated by Westermeier et al. (2006) for *Macrocystis* and *Lessonia*
121 *trabeculata*. One male and one female gametophyte was axenized in July 2002 for long-
122 term maintenance on agar (Müller et al. 2008).

123

124 A sporophyte of *Lessonia berteroana* was collected at Caleta Cisne, Atacama Región, in
125 May 2015. From this specimen two pairs of clonal male and female gametophytes were
126 selected for experiments (Table S1). Additional clonal gametophyte cultures were
127 established from specimens collected in Jan 2017 at the following locations along the
128 Atacama coast ranging from North of the township Caldera to Huasco: Flamenco, Los
129 Médanos, Torres del Inca, Chorillos, Bahía Salado, Caleta Pajonales, Carrizal Bajo, and

130 Ttotal (Fig. 1; Table S1). These strains are maintained in our culture collection, since
131 they represent a first attempt to preserve different genotypes of *L. berteroana* from
132 localities that are presently under severe exploitation. In total, our study included 17 male
133 and 21 female gametophyte clones of *L. berteroana* from 9 localities. Cultures were
134 maintained in 60 mm polystyrene Petri dishes at 10°C, under illumination with 0.5-3 μmol
135 photons $\text{m}^{-2} \text{sec}^{-1}$ (hereafter low light condition) from daylight type fluorescent lamps
136 (Osram DuluxStar Lumilux 11W cool White) for 14h day⁻¹. Agar cultures were transferred
137 in 6 month intervals. Prior to experiments material from agar cultures was transferred to
138 and maintained in liquid culture medium for at least 1 month. Cultures in liquid medium
139 were maintained by bi-weekly transfer intervals. Artificial sea water was prepared from a
140 commercial salt mixture (Tropic Marin Pro-Reef; www.tropic-marin.com) in deionized
141 water, and adjusted to 30 PSU salinity with a sodium chloride refractometer (ATAGO,
142 Sodium Chloride 0-10%). After autoclaving, 20 mL L⁻¹ Provasoli enrichment (Starr and
143 Zeikus 1993) was added.

144

145 In order to induce gametogenesis, suspensions of few-celled gametophyte fragments
146 were prepared by cutting with a scalpel or mild homogenization in a 1.5 mL Eppendorf
147 tube. Exposure in fresh culture medium at 10°C under white light at 40 μmol photons m^{-2}
148 sec^{-1} (hereafter high light condition) resulted in formation of eggs and spermatozoids,
149 followed by fertilization and appearance of juvenile sporophytes. Using this approach we
150 made conspecific and interspecific crosses using one locality representative for each
151 species (Table 1). All unialgal and cross-fertilization experiments were repeated two to
152 three times (otherwise is stated in Table 1). Additionally, we worked with strains from
153 localities distant enough where *Lessonia* species in the intertidal can be recognized as *L.*
154 *berteroana* and *L. spicata* respectively, based on González et al. (2012) observations
155 (see Fig. 1).

156

157 **Results**

158

159 *L. spicata* gametophytes

160 Spore samples from field-specimens of *Lessonia spicata* produced equal numbers of
161 male and female gametophytes with clear dimorphism in cell dimensions and branching
162 pattern (Fig. 2). Under low light conditions they grow in unlimited manner, and
163 reproduction was not triggered whatsoever. In absence of a partner from the opposite
164 sex, gametophytes may remain growing somatically (Fig. 3). In case that eggs/sperm are

165 formed (as in high light treatment), they eventually die. Therefore, sporophytes are not
166 formed from our *L. spicata* unialgal cultures.

167

168 *Lessonia berteroana* gametophytes

169 In principle, gametogenesis and zygote development in *Lessonia berteroana* followed the
170 same development pattern (Fig. 4). However, in contrast to *L. spicata*, female
171 gametophytes of *L. berteroana* from almost all localities showed a tendency to apogamy
172 (Table 1). Apogamic sporophytes emerged from apical as well as intercalary cells of
173 somatic gametophyte filaments, and started to form multicellular foliose sporophyte thalli
174 (Fig. 5). They were variable in cell shapes and growth patterns, but appeared to be fully
175 viable and grew up to several mm in our laboratory conditions. The potential to apogamy
176 seems to vary between localities. We found it in 2 out of 5 female gametophytes from
177 Los Médanos, while in all female gametophyte clones from Flamenco, Chorrillos (Fig. 5),
178 Caleta Cisne, Bahía Salado, C. Pajonales, Carrizal bajo and Totoral (Fig. 6). These
179 strains normally showed massive production of apogamic sporophytes in low light
180 conditions, and account to the 67% of all *L. berteroana* female strains used in this study.

181

182 In addition, some clonal female gametophyte cultures of *L. berteroana* produced eggs,
183 which in the absence of spermatozooids developed to juvenile sporophytes via
184 parthenogenesis (Table 1; Figs. 7 and 8). Female gametophytes from Torres del Inca
185 were especially light-sensitive, and produced eggs and partheno-sporophytes even
186 under low light (Fig. 7). Only two strains from Los Medanos (LM 02 f and LM 05 f)
187 remained growing and lacked any apomixis outcome. On the contrary, all male
188 gametophytes of *L. berteroana* generally showed very slow growth and appeared as
189 compact packages (see Fig. 4). They responded well to gametogenesis conditions,
190 forming dense packets of antheridia releasing spermatozooids. No hints of apomixis were
191 observed for any of them (17 strains; Table S1) in our experimentations.

192

193 *Conspecific crosses*

194 From *L. spicata* crosses, eggs produced zygotic sporophytes in presence of conspecific
195 spermatozooids (Fig. 2). Release of eggs and spermatozooids in *L. spicata* occurred from
196 the sixth day onward. Zygotes immediately started to develop, forming uniseriate, and
197 later multicellular foliose juvenile sporophytes, which grew up to 8 mm under our culture
198 conditions (Fig. 9). *L. spicata* cultures did not show deviations from normal sexual life
199 history. Unfertilized eggs were generally moribund. Occasional cell divisions of
200 unfertilized eggs resulted in irregular cell complexes, but we did not see parthenogenetic

201 nor apogamic development of viable sporophytes (see Fig. 3 for comparison).
202 Consequently, we consider sporophytes resulting from matings of *L. spicata* eggs with *L.*
203 *spicata* spermatozooids (Fig. 9) as heterozygous (Table 1).

204
205 After one attempt to cross *L. berteriana* female and male gametophytes (Fig. 4), we
206 observe predominant apomixis going on even before inducing gametogenesis We did not
207 continue using female gametophytes of *L. berteriana* conspecific crosses onwards,
208 since an unseparable mixture of zygotic and apomictic sporophytes was expected (Table
209 1).

210
211 *Interspecific crosses*
212 Gametophytes of female *L. spicata* from Mar Brava and male *L. berteriana* from Caleta
213 Cisne were mixed in two independent experiments, and subjected to gametogenesis
214 treatment (Table 1). In both reciprocal crosses we observed egg formation and sperm
215 attraction, which normally are used as proxies for fertilization in brown algae. Oogonia
216 and eggs, antheridia and spermatozooids appeared, and at 37 days numerous juvenile
217 sporophytes were recorded, mostly in dense groups emerging from remnants of a female
218 gametophyte in the centre (Fig. 10). These observations were less recurrent in the
219 reciprocal cross *L. bert* CC 11 f x *L. nig* MB 99-61 m, where both parthenogenesis and
220 apogamy are constitutively happening under low and high light regimes. Based on the
221 controlled parameters (e.g. light conditions, temperature, and culture medium) of this
222 experiment we concluded that the resulting sporophytes were true sexual hybrids.

223 224 **Discussion**

225
226 *Apomixis in L. berteriana female gametophytes and its putative contribution to L.*
227 *berteriana ecology*

228 Normally, kelp gametophytes can be maintained for decades as stock cultures in liquid
229 culture medium or in axenic condition on agar, with low temperature and irradiance
230 (Lüning and Neushul 1978). While cultures on agar are routinely transferred twice per
231 year, gametophytes may survive for several years untouched in liquid culture medium
232 (Müller et al. 2008). Typically, these gametophytes may be sensitive at high irradiance
233 levels, and therefore isolation and maintenance at low light/red light is suggested to
234 avoid undesirable fertility (Lüning and Dring 1975). Our *L. berteriana* strains from
235 northern Chile were even more sensitive and became fertile under extremely low light
236 levels, where members of any other Laminariales species, including *L. spicata* and

237 *Macrocystis pyrifera* from several latitudes and heterogeneous climates, remained sterile
238 (Hoffmann and Santelices 1982; Westermeier et al. 2006).

239

240 In land plants it is quite well documented that apomixis is spatially structured, and linked
241 with heterogeneous habitats (Cosendai and Hörandl 2010). The drivers for apomixis are
242 not yet widely explored in seaweeds, but some findings shed light into this question.

243 Marginal populations of *Laminaria digitata* exhibit a higher prevalence of geographical
244 parthenogenesis based on population genetic structures (Oppliger et al. 2014). In our

245 study, a strong tendency for apomixis is particularly characteristic for a significant

246 proportion of *L. berteriana* strains (26 – 28 °S), but non-existent in *L. spicata* (42 °S).

247 This is inconsistent with the observations made by Oppliger et al. (2007). They reported

248 that *Lessonia nigrescens* from Central Chile (Las Cruces, 33°S, *L. spicata* based on

249 current *Lessonia* systematics) is able to produce sporophytes by parthenogenesis, which

250 during early development tended to diploidize. Temporal-fluctuating factors may also

251 affect gametophyte performance. Murúa et al. (2013) determined that fertility in female *L.*

252 *trabeculata* changes dramatically between annual seasons, and even inter-annually after

253 comparing with analogous non-contemporaneous studies. Tatarenkov et al. (2005)

254 found a parthenogenetic dwarf morph of *Fucus vesiculosus*, as the likely result of

255 prolonged exposure to low salinity levels. Genetic/environmental mediation of apomixis

256 has been better studied in plant models. *Eragrostis curvula* (weeping lovegrass)

257 distributes in biotypes that may be obligate or facultatively apomictic (Voigt and Bashaw

258 1976). Sexual diploids are rare (Voigt 1971); however, towards later generations some

259 purely sexual individuals reformed into apomictic, which involved genetic and epigenetic

260 changes (Zappacosta et al. 2015). We therefore cannot rule out that apomixis in *L.*

261 *berteriana* may be linked with our focalized sampling (majorly in 2017), and that both

262 gametophyte viability and apomictic deviations are genetic and/or environmentally-

263 driven.

264

265 Kelp asexuality in natural populations is a topic poorly integrated in seaweed ecology,

266 but has demonstrated to be more common and relevant than previously thought. In

267 *Ecklonia radiata* two morphs have been identified with contrasting different reproductive

268 strategies, one of them vegetative (Coleman and Wernberg 2018). As in plants, in *E.*

269 *radiata* asexuality contributes to fix phenotypes and genotypes (especially

270 heterozygous). Asexual reproduction has often been correlated with range expansions

271 (Krueger-Hadfield et al. 2016), substrate colonization (Westermeier et al. 2016),

272 senescence recovery (Murúa et al. 2017), and better acclimation under unfavourable

273 conditions (Demes and Graham 2011). For plants, apomictic reproduction leading to
274 healthy progeny indeed offers ecological advantages that can be extrapolated to
275 *Lessonia*, such as reduction of costs for male sexual selection and outcrossing sex
276 (reviewed in Richards 2003). In our culture experiments, female gametophytes of
277 *Lessonia berteroa* were able to perform both apogamy and parthenogenesis
278 deviations. In consequence, the sporophytes produced by a bisexual mixture of
279 gametophytes is expected to contain regular diploid heterozygous as well as genetically
280 female apomictic sporophytes. A high apomictic tendency in gametophytes may reflect
281 the results observed by Tellier et al. (2011), who pointed out that genetic diversity (e.g.
282 allele richness, gene diversity) is significantly more reduced in *L. berteroa* than *L.*
283 *spicata*, suggestive of different histories between populations. Vegetative propagation
284 from big sporophytes has not been reported in *Lessonia* like in other kelps; therefore, a
285 higher apomixis extent provides a probable hypothesis to explain such low levels of
286 genetic diversity. The consequences for such particular development need to be
287 elucidated.

288

289 *L. berteroa* cross-fertilization with *L. spicata*

290 Based on molecular and morphological evidence, González et al. (2012) sub-divided the
291 former taxon *Lessonia nigrescens* into two separate species: *L. spicata* and *L.*
292 *berteroa*. In parallel, Tellier et al. (2011) studied 12 field populations along 50 km of
293 the contact zone between the two taxa using molecular markers. They reported complete
294 reproductive isolation with strict geographic segregation and total lack of spatial co-
295 existence. The factors responsible for this rigid separation in the natural habitat are
296 unknown. On laboratory scale, however, our study showed ambiguous results: the two
297 species *L. spicata* and *L. berteroa* are sexually compatible, and we are sure that at
298 least in one direction (with *L. spicata* as female partner) 100% of the obtained progeny
299 were viable hybrids. Gamete interaction and recognition mechanisms worked perfectly,
300 up to the production of almost cm-size sporophytes. Hybridization in kelps with
301 contrasting phenotypes/genotypes and well-delimited separation in natural habitats has
302 also been studied for the two ecomorphs of *Macrocystis pyrifera* (Westermeier et al.
303 2007). Since later studies demonstrated that they belong to the same species (Macaya
304 and Zuccarello 2010), the factors regulating such spatial separation are unknown. Our
305 study may indicate that two *Lessonia* taxa are phylogenetically close and/or their cell
306 architecture/molecular clues are similar enough to make them sexually compatible,
307 allowing cross-fertilization and production of viable hybrid sporophytes at least under
308 laboratory conditions.

309

310 *Implications of apomixis and hybrid viability for Lessonia fishery and breeding programs*

311 As asexual reproduction strategies in natural kelp populations are starting to be
312 documented, its erroneous prevalence estimation in the field may conduct to design
313 inaccurate policies for kelp management. This ultimately may lead to negative
314 consequences for the fishery/ecology of highly exploited resources, such as *L.*
315 *berteroana* and *L. spicata*. As pointed out before, populations dominated by asexual
316 propagation may perform better. However, this is not always the case and sometimes
317 asexuality led to maladaptation (Oppliger et al. 2014). How these processes are now
318 varying in the current climate change scenario is virtually unexplored, even for higher
319 plants (Walther et al. 2009).

320

321 In sharp contrast, apomixis is a potent tool in modern agronomy of terrestrial seed plants,
322 stabilizing desirable phenotypes (Barcaccia and Albertini 2013). Particularly in seaweeds
323 has also been suggested to facilitate mariculture in the kelp *Undaria pinnatifida* (Shan et
324 al. 2013), whereby parthenosporophytes showed high attachment to culture ropes and
325 more attractive yields. Our study opens the chance to generate apomictic kelp seedlings
326 of selected stable genotypes. It also provides materials and knowledge for pilot scale
327 mariculture experiments making use of Mendelian genetics including heterosis breeding.
328 The limitation of our observations to laboratory scale, does not allow to judge on the
329 survival potential of apomictic sporophytes in the natural habitat. However, our study
330 presents a well-defined basis for a search for factors supporting species segregation in
331 the natural habitat. We plan to grow up interspecific hybrid sporophytes *L. spicata* female
332 x *L. berteroana* male to maturity in our pilot scale mariculture facilities. Jointly with a
333 study using molecular sex markers, this strategy will offer a chance to verify their
334 heterozygous character, determine if our *Lessonia* sporophytes may be product of
335 pseudogamous and/or autonomous apomixis and to identify factors such as meiotic
336 failure responsible for the observed species separation in the natural habitat.

337

338 Figure Legends

339

340 Fig. 1. Coast of Chile showing collection sites of *Lessonia berteroana* in northern Chile,
341 ranging from North of the townships Caldera to Huasco in the South, and of *L. spicata* in
342 Chiloé (For strain details see Table S1). The distribution of both species (after Gonzalez
343 et al. 2012) is indicated in coloured rectangles in the left side.

344

345 Figs. 2-10. Diversity of reproductive outcomes in *Lessonia* gametophytes. 2: Mixed
346 sexually mature female and male gametophytes of *L. spicata* (L nig MB 97-23-4-f x L nig
347 MB 99-61 m) after 6 days. Open arrowheads: antheridia; solid arrowheads: zygotes
348 developing on oogonium apertures. 3: Solitary female gametophyte of *L. spicata* (L nig
349 MB 97-23-4-f) derived from axenic agar culture after 37 days under gametogenesis-
350 inducing conditions at high light. No apomictic sporophytes present. 4: *L. berteriana*
351 conspecific cross from Caleta Cisne (L bert CC 11 f x L bert CC 7 m) after 13 days of
352 culture. 5: Early apogamic sporophyte (arrow) developing from a somatic female
353 gametophyte cell of *L. berteriana* from Chorrillos (L bert Cho 05 f) at low light intensity
354 (Image from stock culture). 6: Multiple apogamic sporophytes developing on a female
355 gametophyte of *L. berteriana* from Caleta Totoral (L bert CT 01 f) at low light intensity
356 (Image from stock culture). 7: Solitary clonal female gametophyte of *L. berteriana* from
357 Torres del Inca (L bert TI 1 f), forming oogonia, eggs (double arrowheads) and apomictic
358 sporophytes at reduced light intensity (Image from stock culture). 8: Parthenogenetic
359 sporophyte originating from an un-fertilized eggs of a clonal female gametophyte of *L.*
360 *berteriana* from Caleta Cisne (L bert CC 11 f), 38 days after the parent was transferred
361 from low to high light. 9 and 10: Parallel batches of female *L. spicata* at 37 days
362 producing zygotic sporophytes in presence of fertile male gametophytes. 9: conspecific
363 cross *L. spicata* (L nig MB 97-23-4-f x L nig MB 99-61 m). 10: hybrid cross *L. spicata* x *L.*
364 *berteriana* (L nig MB 97-23-4-f x L bert CC 7 m). Scale bars: 100 μ m for Figs. 2, 4 – 8
365 and 0.5 mm for Figs. 3, 9 and 10.

366

367

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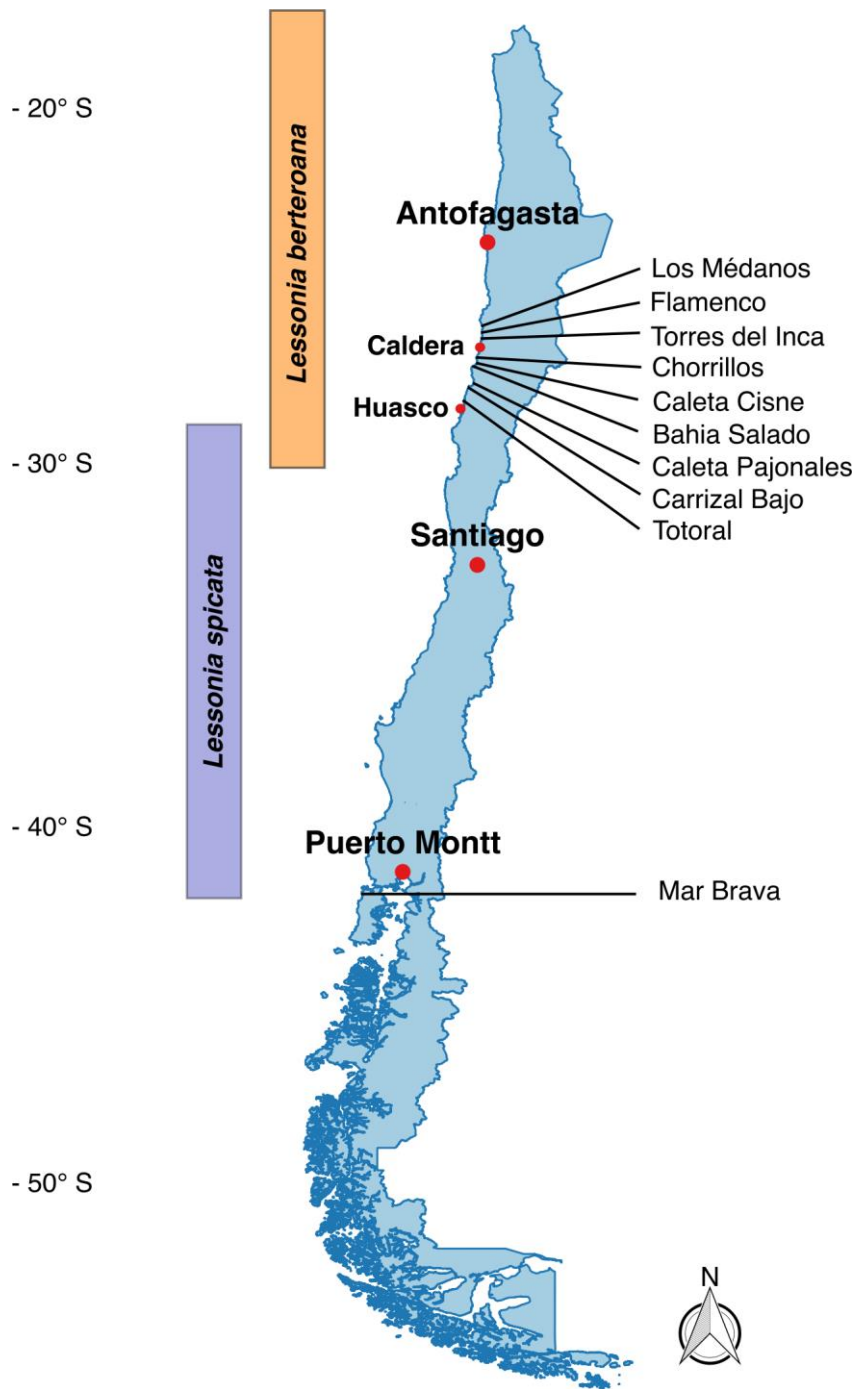
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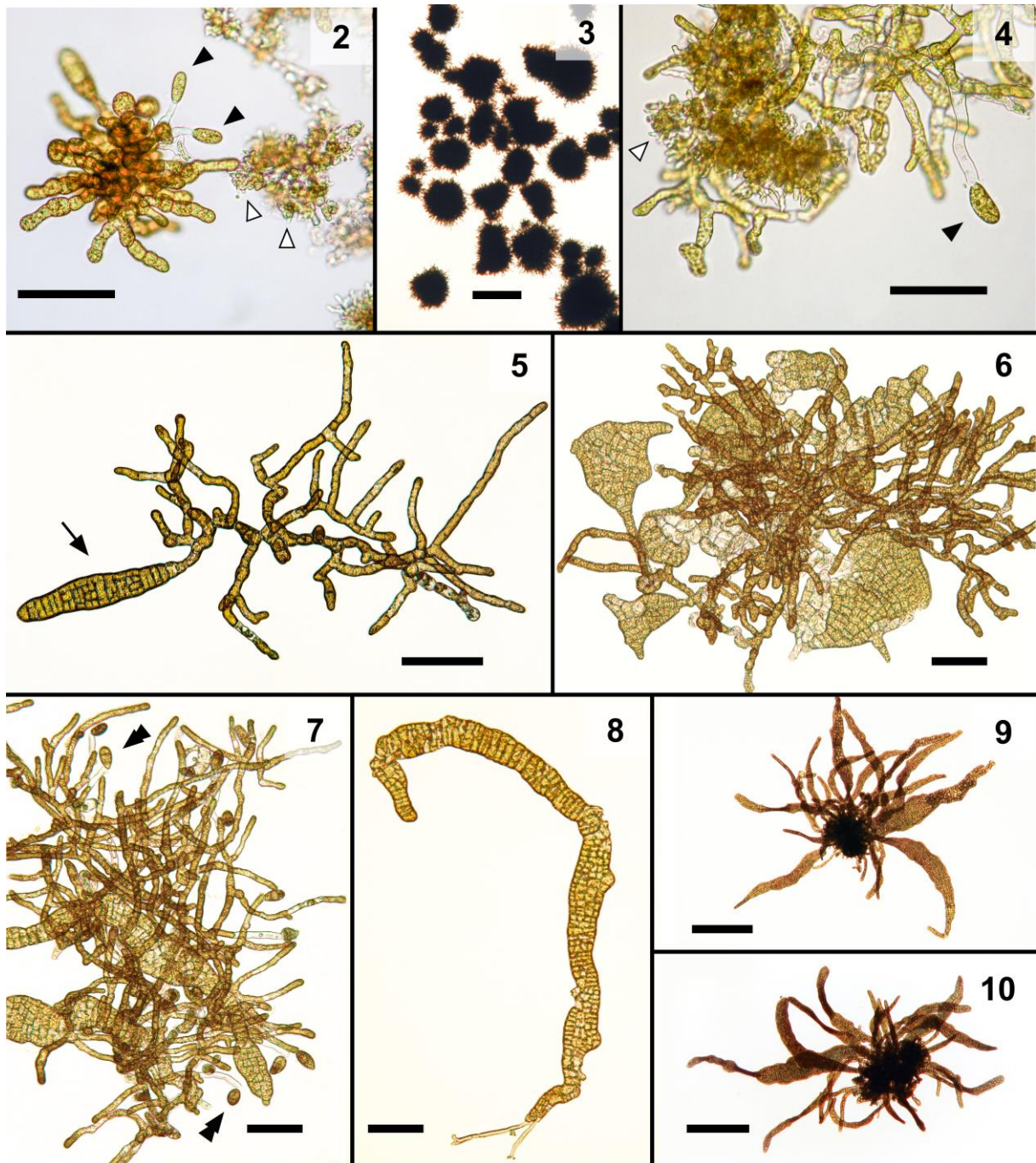
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496 Fig. 1
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501 Figs. 2-10
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Table 1. Summary of outcomes after cultivating *Lessonia* unisexual strains and intra-/inter-specific crosses, under low light (LL) and high light (HL) regimes.

Unisexual strains	LL	HL
L nig MB 99-61-m	Sg (65d/X)	Sm (65d/X)
L nig MB 97-23-4-f	Sg (65d/X)	Sg/Sm (65d/X)
All male L bert	Sg (65d/X)	Sm (65d/X)
L bert CC 11 f	As (81d/-)	Ps/As (81d/5mm)
L bert LM 05 f	Sg (81d/X)	-
L bert LM 02 f	Sg (81d/X)	-
L bert TI 1 f	Ps (81d/-)	-
L bert TI 2 f	Ps (81d/-)	-
All remaining female L bert	As (81d/-)	-
Crosses	LL	HL
L nig MB 97-23-4-f x L nig MB 99-61 m	-	Hs (60d/8mm)
L bert CC 11 f x L bert CC 7 m *	-	Ps/As/Hs? (13d/-)
L bert CC 11 f x L nig MB 99-61 m	-	Ps?/As?/Hs (78d/7mm)
L nig MB 97-23-4-f x L bert CC 7 m	-	Hs (37d/4mm)

Sg: somatic growth

Sm: sexual maturity (release of eggs/spermatozoids and die off later because of unsuccessful mating)

Ps: parthenosporophytes

As: apogamic sporophytes

Hs: heterozygotic sporophytes

- not tested/not quantified

() days for diagnosis/maximum size for the largest sporophyte (X: not applicable)

* Carried out once