

Spore output in selected species of Lejeuneaceae (Marchantiophyta) from China

Qiong HE & Rui-Liang ZHU*

Department of Biology, School of Life Science, East China Normal University,
3663 Zhong Shan North Road, Shanghai 200062, China

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Abstract – The spore output of 26 selected species of 11 genera of Lejeuneaceae was investigated by the direct artificial counting method. The mean spore output per capsule ranges from 257 in *Cololejeunea magnilobula* to 5038 in *Ptychanthus striatus*. Compared with other leafy liverwort families, Lejeuneaceae have a much lower and more stable spore output. Out of eight species of Ptychanthoideae, *Acrolejeunea pusilla* is the only species with a mean spore output of less than 1000 spores per capsule. *Acrolejeunea securifolia*, *Cheilolejeunea larsenii* and *Cololejeunea furcilibulata* are reported for China for the first time.

***Acrolejeunea securifolia* / *Cheilolejeunea larsenii* / *Cololejeunea furcilibulata* / endangered liverworts / Hepaticae / reproductive biology**

INTRODUCTION

The Lejeuneaceae are the largest family of liverworts (Marchantiophyta) with about 1700 currently accepted species in 81 genera. Many species of Lejeuneaceae are minute and considered to be rather difficult taxonomically. Data on spore production are a prerequisite for the study of population maintenance, spore dispersal, life-cycle analysis, classification and phylogenetic analysis (Bisang, 2001). Although there is some information on the spore output of nearly 100 bryophytes (mainly mosses and thalloid liverworts) (Longton & Schuster, 1983; Longton, 1997; Vanderpoorten & Goffinet, 2009; He & Zhu, 2010), the spore output in Lejeuneaceae is still extremely poorly known. The significance and taxonomic value of spore output in the intergeneric and infrageneric divisions of Lejeuneaceae have also not been investigated. This paper presents our results on spore output of 26 species belonging to 11 genera of Lejeuneaceae from China, and aims to provide the basic data of spore production of Lejeuneaceae.

* Correspondence and reprints: lejeunea@163.com

MATERIALS AND METHODS

The well developed, undehisced mature capsules of select liverworts were collected in China during 2008-2010. For accuracy, spore production of all species was counted in a direct way (direct artificial counting method). Ten well developed, unopened mature capsules were chosen randomly for direct artificial counting. Each capsule was opened and the spores were dispersed evenly in glycerin by careful dissection under an advanced dissecting microscope (Zeiss V8). The prepared slides were observed and the spore outputs were counted using light microscopy (Olympus microscope, BX 41). Spore outputs of *Cheilolejeunea xanthocarpa*, *Mastigolejeunea indica*, *Ptychanthus striatus*, and *Trocholejeunea sandvicensis* were also estimated by hemacytometer counts in 10% sucrose solution as previously described by He & Zhu (2010). All voucher specimens are listed in the appendix of the present paper.

RESULTS AND DISCUSSION

The spore production in bryophytes is usually huge, and was usually estimated by hemacytometer counts (Ingold, 1959; He & Zhu, 2010). In the present study the spore output of 26 selected species of Lejeuneaceae was investigated by the artificial counting method. For method's comparison, we also used hemacytometer counts to estimate the spore output of *Cheilolejeunea xanthocarpa*, *Mastigolejeunea indica*, *Ptychanthus striatus*, and *Trocholejeunea sandvicensis*. The results obtained by the direct artificial counting method are slightly higher than those by hemacytometer counts (Table 1).

The spore output of 25 species of Lejeuneaceae is here reported for the first time; spore counts have already been published for *Trocholejeunea sandvicensis* (He & Zhu, 2010). The mean spore output per capsule ranges from 257 in *Cololejeunea magnilobula* to 5038 in *Ptychanthus striatus* (Table 1). Out of 26 species investigated here, 20 species (76.9%) have mean values of 500-3000 per capsule, and values of less than 500 occurred only in five species (19.2%), namely *Cheilolejeunea intertexta*, *Cololejeunea magnilobula*, *C. ocelloides*, *Colura tenuicornis*, and *Microlejeunea punctiformis*. *Ptychanthus striatus* showed the highest spore output of up to 5110 by the artificial counting method, but 5750 by hemacytometer counts. Out of eight species of Ptychanthoideae, *Acrolejeunea pusilla* is the only species with a spore output of less than 1000. In Lejeuneoideae *Cheilolejeunea*, *Cololejeunea* (except for *C. trichomanis*), *Colura* and *Microlejeunea* had a much lower spore output than most species of *Lejeunea* (Table 1). All investigated *Lejeunea* species had values of more than 1000 spores per capsule (1061-2610 spores per capsule), with the exception of *Lejeunea flava* with a value of only 574. Whether *Microlejeunea* is a separate genus from *Lejeunea* remains controversial (Schuster, 2001; Zhu & So, 2001; Crandall-Stotler *et al.*, 2009). However, *Microlejeunea punctiformis* has the lowest value of spore production (only 270 per capsule) of all *Lejeunea* and *Microlejeunea* species investigated here. *Cheilolejeunea xanthocarpa* has traditionally been recognized as *Leucolejeunea xanthocarpa* (Malombe, 2009). Its spore output is in the range of typical species of *Cheilolejeunea* such as *C. intertexta*, *C. larsenii*, and *C. trapezia*.

Table 1. Spore output per capsule in 26 selected species of Lejeuneaceae from China

Species	Mean spore output per capsule (ranging values), obtained by direct artificial counting method	Mean spore output per capsule (ranging values), obtained by hemacytometer counts (only in four species)
<i>Acrolejeunea pusilla</i>	538 (468-646)	–
<i>Acrolejeunea securifolia</i>	1107 (967-1309)	–
<i>Archilejeunea planiuscula</i>	2062 (1858-2405)	–
<i>Cheilolejeunea gaoi</i>	799 (795-804)	–
<i>Cheilolejeunea intertexta</i>	466 (411-538)	–
<i>Cheilolejeunea larsenii</i>	624 (571-665)	–
<i>Cheilolejeunea trapezia</i>	569 (467-627)	–
<i>Cheilolejeunea xanthocarpa</i>	622 (565-663)	562 (406-719)
<i>Cololejeunea furcilibulata</i>	722 (625-855)	–
<i>Cololejeunea magnilobula</i>	257 (212-341)	–
<i>Cololejeunea ocelloides</i>	463 (350-555)	–
<i>Cololejeunea ornata</i>	929 (859-1017)	–
<i>Cololejeunea raduliloba</i>	650 (614-676)	–
<i>Cololejeunea spinosa</i>	926 (833-1076)	–
<i>Cololejeunea trichomanis</i>	1091 (1021-1166)	–
<i>Colura tenuicornis</i>	427 (402-446)	–
<i>Lejeunea anisophylla</i>	2610 (2492-2844)	–
<i>Lejeunea apiculata</i>	2301 (1995-2593)	–
<i>Lejeunea bidentula</i>	1061 (891-1254)	–
<i>Lejeunea flava</i>	574 (501-647)	–
<i>Lejeunea obscura</i>	1626 (1334-1927)	–
<i>Lopholejeunea nigricans</i>	1065 (936-1254)	–
<i>Mastigolejeunea indica</i>	1575 (1472-1685)	1396 (813-2313)
<i>Microlejeunea punctiformis</i>	270 (259-276)	–
<i>Ptychanthus striatus</i>	5038 (4996-5110)	5000 (4250-5750)
<i>Trocholejeunea sandvicensis</i>	1485 (1272-1704)	1450 (728-2850)

Twenty five of the investigated 26 Lejeuneaceae species produce less than 3000 spores per capsule. Although the spore output of only a few leafy liverworts is known, the preliminary results indicate that Lejeuneaceae have a much lower and more stable spore output than other leafy liverwort families such as Anastrophyllaceae (42000 in *Anastrophyllum hellerianum*, Pohjamo & Laaka-Lindberg, 2003; 115000 in *Barbilophozia attenuata*, Jonsson & Söderström, 1988), Cephaloziellaceae (14000-23750 in *Cephaloziella varians*, Smith & Convey, 2002), Lophocoleaceae (23900 in *Lophocolea cuspidata*, Schuster, 1966; 93000 in *L. heterophylla*, Jonsson & Söderström, 1988), Scapaniaceae (1000000 in *Scapania undulata*, 400000 in *Diplophyllum albicans*, Schuster, 1966; 63000-86000 in *Lophozia*, Jonsson & Söderström, 1988), Jungermanniaceae (84150 in *Jungermannia truncata*, He & Zhu, 2010), and Ptilidiaceae (27400 in *Ptilidium pulcherrimum*, Jonsson & Söderström, 1988).

However, the observed tendencies need to be verified by a broader sampling of leafy liverworts, especially Lejeuneaceae.

Fieldwork related to our study led also to three new country records of Lejeuneaceae species: The Asian-Oceanic *Acrolejeunea securifolia* (Gradstein, 1975), the Thailand endemic *Cheilelejeunea larsenii* (Hattori & Mizutani, 1969), and the Indian – tropical African *Cololejeunea furciculobulata* (Wigginton & Grolle, 1996; Asthana & Srivastava, 2003; Asthana & Shukla, 2010) are newly recovered elements of the Chinese liverwort flora.

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APPENDIX

Specimens used in this study.

A species name is followed by the locality and collection. All specimens are housed in HSNU.

Acrolejeunea pusilla (Steph.) Grolle *et* Gradst., CHINA, Fujian, Dehua Co., Wucuo, 25°32'639"N, 118°17'461"E, 303 m, on tree trunk, *Zhu et al. 20100405-5*.

Acrolejeunea securifolia (Nees) Steph. *ex* Watts, CHINA, Guizhou, Leigongshan Nature Reserve, 26°22'163"N, 108°08'994"E, 964 m, on tree trunks, *Zhu et al. 20100826-104*.

Archilejeunea planiuscula (Mitt.) Steph., CHINA, Guangxi, Shiwandashan National Forest Park, 21°53'803"N, 107°54'275"E, 377 m, on tree root, *Ye & Wei 20090715-43*.

Cheilolejeunea gaoi R.L. *Zhu et al.*, CHINA, Guangxi, Shiwandashan National Forest Park, 21°53'611"N, 107°54'568"E, 406 m, on tree base, *Zhu et al. 20100822-15*.

Cheilolejeunea intertexta (Lindenb.) Steph., CHINA, Guangxi, Shiwandashan National Forest Park, 21°54'188"N, 107°54'233"E, 299 m, on tree base, *Wei 20100210-17*.

Cheilolejeunea larsenii Mizut., CHINA, Hainan, Jianfengling Nature Reserve, 18°42'570"N, 108°52'259"E, 880 m, on tree base, *Yu & Peng 20100715-6*.

Cheilolejeunea trapezia (Nees) Kachroo *et* R.M. Schust., CHINA, Guizhou, Fanjingshan Nature Reserve, 27°54'712"N, 108°39'379"E, 1950 m, on tree trunk, *Peng 20100517-6A*.

Cheilolejeunea xanthocarpa (Lehm. *et* Lindenb.) Malombe, CHINA, Fujian, Dehua Co., Shiniushan National Forest Park, 25°37'310"N, 118°28'239"E, 1666 m, on tree trunk, *Zhu et al. 20100403-79*.

Cololejeunea furcilobulata (Berrie *et* E.W. Jones) R.M.Schust., CHINA, Guangxi, Nonggang Nature Reserve, 22°27'808"N, 106°58'115"E, 187 m, on liana, *Wei & Peng 20100920-30*.

Cololejeunea magnilobula (Horik.) S. Hatt., CHINA, Guizhou, Leigongshan Nature Reserve, 26°23'329 N, 108°11'925 E, 2090 m, on dead branches of shrubs, *Zhu et al 20100826-68*.

Cololejeunea ocelloides (Horik.) Mizut., CHINA, Guangxi, Shiwandashan National Forest Park, 21°54'245 N, 107°54'228 E, 290 m, epiphyllous, *Ye & Wei 20090715-45B*.

Cololejeunea ornata A. Evans, CHINA, Zhejiang, Shengzhou, 29°21'08.34 N, 120°36'45.17 E, 416 m, on dead branches, *Zhu 20100216-1*.

Cololejeunea raduliloba Steph., CHINA, Guangxi, Jingxi Co., between Bangliangcun and Minmacun, on decaying logs, *Wei & Peng 20100912-36A*.

Cololejeunea spinosa (Horik.) Pandé *et* Misra, CHINA, Zhejiang, Tianmushan Nature Reserve, 30°19'161"N, 119°26'678"E, 351 m, on tree bark, *Zhu et al.* 20100421-29.

Cololejeunea trichomanis (Gottsche) Steph., CHINA, Guangxi, Shiwandashan National Forest Park, 21°53'564"N, 107°54'471"E, 411 m, epiphyllous, *Zhu et al.* 20100822-26.

Colura tenuicornis (A. Evans) Steph., CHINA, Guizhou, Leigongshan Nature Reserve, 26°23'258"N, 108°11'926" E, 2085 m, on dead branches of shrubs, *Zhu et al.* 20100826-55A.

Lejeunea anisophylla Mont., CHINA, Guizhou, Maolan Nature Reserve, 25°7'120"N, 107°56'446"E, 745 m, epiphyllous, *Peng* 20100520-38A.

Lejeunea apiculata Sande Lac., CHINA, Hainan, Jianfengling Nature Reserve, 18°43'693"N, 108°52'293"E, 978 m, epiphyllous, *Yu & Peng* 20100714-16.

Lejeunea bidentula Herzog, CHINA, Yunnan, Baoshan City, on a hill, 25°05'677"N, 99°19'980"E, 1885 m, on shrub, *Yu* 20100916-5.

Lejeunea flava (Sw.) Nees, CHINA, Guangxi, Shiwandashan National Forest Park, 21°52'818"N, 107°55'058"E, 838 m, on liana, *Wei* 20100222-73.

Lejeunea obscura Mitt., CHINA, Hainan, Wuzhishan Nature Reserve, 18°54'344"N, 109°40'913"E, 770 m, epiphyllous, *Yu & Peng* 20100719-50.

Lopholejeunea nigricans (Lindenb.) Schiffn., CHINA, Guizhou, Maolan Nature Reserve, 25°15'933"N, 108°67'669"E, 520 m, on tree trunk, *Peng* 20100522-25B.

Mastigolejeunea indica Steph., CHINA, Guizhou, Maolan Nature Reserve, 25°17'337"N, 108°04'104"E, 506 m, on wet rock, *Peng* 20100522-2.

Microlejeunea punctiformis (Taylor) Steph., CHINA, Guangxi, Shangsi Co., Pinglongshan, 21°52'000"N, 107°51'277"E, 355 m, on tree trunk, *Zhu et al.* 20100821-6.

Ptychanthus striatus (Lehm. *et* Lindenb.) Nees, CHINA, Guangxi, Nonggang Nature Reserve, 22°27'808"N, 106°58'115"E, 187 m, on rock, *Wei & Peng* 20100920-24.

Trocholejeunea sandvicensis (Gottsche) Mizut., CHINA, Zhejiang, Linan, Tianmushan Nature Reserve, on tree trunk, *Zhu* 20080422-32.