PERSPECTIVES IN
Biodiversity
VOLUME 3 / ISSUE 1 / 2025

Published ahead of issue
Received: 14 August 2025
Accepted: 15 August 2025
Published: October 2025

Using ascospore size as a first tool to identify verrucoid *Pertusaria* DC. species from Aotearoa / New Zealand

Anthony E. Aldridge, Jennifer M. Bannister

https://doi.org/10.34074/pibdiv.003108

This publication may be cited as: Aldridge, A. E., Bannister, J. M. (2025). Using ascospore size as a first tool to identify verrucoid *Pertusaria* DC. species from Aotearoa / New Zealand. *Perspectives in Biodiversity*, 3(1): 83–98.

Contact:
www.unitec.ac.nz/epress
ePress
Unitec
Private Bag 92025, Victoria Street West
Tāmaki Makaurau Auckland 1142
Aotearoa New Zealand

Unitec is a business division of Te Pūkenga –
New Zealand Institute of Skills and Technology

ISSN 3021–114X

Using ascospore size as a first tool to identify verrucoid *Pertusaria* DC. species from Aotearoa / New Zealand

Anthony E. Aldridge[1]*, Jennifer M. Bannister[2]

Affiliations:
1. **20 Genia Drive, Wakefield, Tasman 7025, New Zealand**
2. **Department of Botany, Otago University, 464 Great King Street, Dunedin 9016, New Zealand**

* **Corresponding author: tony@southnet.co.nz**

Senior Editor: Professor Peter J. de Lange
Article type: Research paper

Abstract

The widespread crustose lichen genus *Pertusaria* DC. (Pertusariaceae) is present throughout Aotearoa / New Zealand. These *Pertusaria* species exhibit many variable thalline features in colour and shape. The chemicals present are helpful in the identification of *Pertusaria* species, but the very detailed results obtained by high-performance liquid chromatography (HPLC) now render existing descriptions difficult to use. The average spore size, substrate and number of spores per ascus, however, remain stable features amenable to statistical methods for discriminating among species. In this study, the length and width of 563 ascospores of 23 species of verrucoid *Pertusaria* found in Aotearoa / New Zealand were measured. Fresh ascospore preparations were used and only mature ascospores were measured. All measurements were made by one person (JMB). Published ascospore measurements were later added so that the results would include information about all Aotearoa / New Zealand verrucoid species of *Pertusaria*. Statistical outline analysis showed that mature ascospores were consistently elliptical in 2D ($R^2 > 99\%$), confirming that length and width are sufficient to completely describe ascospore shape. Bivariate analysis highlighted which species were significantly different from each other. This novel, yet simple visual display of mean ascospore length and width, together with the type of substrate and the number of ascus spores, is proposed as an effective means of identifying verrucoid species of *Pertusaria*.

Keywords

Logarithmic transformation, resistant estimators, confidence regions, discoid, meiosis, *Pertusaria*, Aotearoa / New Zealand

Introduction

The genus *Pertusaria* DC. (Pertusariaceae, lichenised Ascomycota) is a large cosmopolitan crustose lichen genus that occurs on a wide range of substrates. It is found throughout Aotearoa / New Zealand, occupying habitats ranging from coastal to subalpine, mainly on the bark of various trees both indigenous and introduced and on a range of rock types, and offshore, from the Kermadec Islands to the Subantarctic Islands. *Flora of New Zealand Lichens* lists over 50 species of *Pertusaria* (Galloway 2007). Since 2007 some of these species have been reassigned to other genera: *P. jamesii* Kantvilas is now *Gyalectaria jamesii* (Kantvilas) Schmitt, Kalb & Lumbsch (Schmitt et al. 2010), *P. velata* (Turner) Nyl. is now *Varicellaria velata* (Turner) Schmitt & Lumbsch (Schmitt et al. 2012), and several species have been transferred to *Lepra* Scop. (Wei et al. 2017). A specific name has changed; *P. melanospora* Nyl. is now referred to as *P. crassilabra* Müll.Arg. (Kantvilas 2018). One species, *P. macloviana* Müll.Arg., has been deleted as it was found to be a misidentified specimen, and this was reidentified as *P. stellata* Fryday (Fryday 2008), a new discoid species for Aotearoa / New Zealand. Other new species or new records have been published: *P. allosorodes* Elix & A.W.Archer (Archer & Elix 2013), *P. endoxantha* Vain. (Marshall & Blanchon 2017), *P. flavoexpansa* Kantivilas & Elix (Ludwig & Kantvilas 2015), *P. puffina* A.W.Archer & Elix (Er et al. 2015), *P. southlandica* A.Knight, Elix & A.W.Archer (Knight et al. 2011) and *P. dennistonensis* Elix & A.W.Archer (Elix & Archer 2007). Additionally, *P. wirthii* Elix & A.W.Archer (Archer & Elix 2013) was moved to *Lepra wirthii* (Elix & A.W. Archer) Schmitt, B.P. Hodk. & Lumbsch (Wei et al. 2017).

The ascospores of *Pertusaria* are produced in apothecia, and either manifest as flat discs (discoid) or are immersed in thalline warts called verrucae that reach the exterior via small openings called ostioles. The verrucoid species studied in this paper are listed in Tables 1 and 2.

The asci of *Pertusaria* produce different numbers of ascospores according to the species: one, two, four, or five to eight. In the young ascus the fusion nucleus divides meiotically to produce four haploid nuclei; this is the first meiotic division. In many species a second meiotic division gives a total of eight haploid nuclei. Of those asci that have only the first meiotic division, not all four nuclei may produce ascospores – one, two, or four are common numbers. Those species with the second mitotic division usually produce eight ascospores, although a few ascospores may be aborted. The number of ascospores produced varies with the species, indicating a genetic control (Erbisch 1969; Honegger & Scherrer 2008). Discharge of the ascospores from the verrucae occurs after rainfall, when the jelly-like contents of the apothecia absorb water and swell, forcing mature asci to open and release the ascospores through the apothecial openings, the ostioles. The larger spores can often be seen lying on the thallus outside the ostioles. Because the ascospores ooze out of the ostioles, the aerodynamic features that might be found in those released as projectiles are not needed, while a larger size may give an advantage in germination and the establishment of a new thallus (Pyatt 1973; Sweetwood et al. 2012).

While identifying Aotearoa / New Zealand *Pertusaria* specimens, it became obvious that many features used for identification were variable. *Pertusaria* thalli of the same species can differ in appearance and colour. The number and size of ostioles in a verruca are not reliable features to separate *Pertusaria* species. The ostioles in young verrucae can be minute, but as the apothecia within the verrucae produce mature spores, the ostioles can enlarge and in some cases merge. The presence and type of lichen substances are not always easy to detect using spot tests, and some cannot be detected in this way. An ultraviolet lamp can detect the presence of xanthones, but not the actual xanthone. Definitive testing often requires HPLC, or the less detailed thin-layer chromatography (TLC), both necessitating laboratory space and a source of funding. Standard microscopic examination shows two striking features of *Pertusaria* that are relatively straightforward to measure, and each with a direct link to reproduction that might also reflect the possible genetic and evolutionary directions noted by Erbisch (1969): one is the number of ascospores in an ascus (one, two, four, or five to eight), and the other is the order of magnitude change in spore length (30 to 300 µm). We aim to report how ascospore shape can be quantified and how, through the order of magnitude range in ascospore size together with the number of ascospores in the ascus and substrate, we are able to discriminate *Pertusaria* species.

Methods

In this study, 23 species of verrucoid *Pertusaria* were examined from recent collections (Table 1). They were identified using keys and written descriptions from

Table 1. Aotearoa / New Zealand verrucoid *Pertusaria* species that were measured in this study to estimate mean ascospore length and width (N is the number of measured ascospores).

Species	Substrate	Spores in ascus	N	Mean length (μm)	Mean width (μm)
P. albissima Müll.Arg.	Corticolous	8	10	64.5	31.5
P. alboatra Zahlbr.	Corticolous	2	37	86.0	33.0
P. allosorodes Elix & A.W.Archer	Corticolous	2	15	214.0	68.0
P. bartlettii A.W.Archer & Elix	Corticolous	8	17	63.0	30.0
P. endoxantha Vain.	Corticolous	8	35	87.0	38.0
P. erumpescens Nyl.	Corticolous	8	20	37.0	19.0
P. graphica C.Knight	Saxicolous	4	54	69.0	35.0
P. hadrospora A.W.Archer & Elix	Corticolous	2	11	230.0	54.0
P. hypoxantha Malme	Saxicolous	8	26	59.5	32.0
P. knightiana Müll.Arg.	Saxicolous	2	23	157.5	62.0
P. laevis C.Knight	Corticolous	8	16	43.0	21.0
P. lavata Müll.Arg.	Saxicolous	8	14	87.0	35.0
P. lophocarpa Müll.Arg.	Saxicolous	8	24	50.0	28.0
P. melaleucoides Müll.Arg.	Corticolous	2	6	93.4	40.0
P. otagoana D.J.Galloway	Saxicolous	1	34	256.0	75.0
P. parvula A.W.Archer & Elix	Corticolous	8	6	38.0	19.5
P. sorodes Sirt.	Corticolous	2	69	203.0	67.0
P. southlandica A.Knight, Elix & A.W.Archer	Corticolous	2	5	112.0	45.0
P. spilota A.W.Archer & Malcolm	Corticolous	8	10	49.5	25.0
P. subplanaica A.W.Archer & Elix	Corticolous	8	28	96.0	37.0
P. subverrucosa Nyl.	Saxicolous	2	60	171.5	60.0
P. theochroa Kremp.	Corticolous	8	21	85.0	31.0
P. thiospoda C.Knight	Corticolous	2	22	111.0	42.0
		Total	563		

Galloway (2007), Archer (2012), and Archer and Elix (2018). Information about three verrucoid species added to the Aotearoa / New Zealand list of *Pertusaria* species since 2007 was obtained from the published papers for *P. allosorodes*, *P. endoxantha* and *P. southlandica*.

Spores were obtained by cutting thin longitudinal sections from the verrucae and placing these in a drop of 10% potassium hydroxide (KOH) on a microscope slide and adding a coverslip. After a short interval, light pressure was applied to the coverslip to spread out the softened material. Freshly prepared slides were examined using a Leica DM 1000 microscope fitted with a Leica K3 camera. Photographs and ascospore measurements were made using Leica LASX software. The length and width of all mature ascospores released from the asci in each preparation were measured and recorded. The ascospore numbers for each species were from more than one specimen. The number of ascospores per ascus was also recorded.

A total of 563 ascospores were measured for basic morphometric parameters of length and width. Additionally, 69 different ascospores were photographed

Table 2. Aotearoa / New Zealand verrucoid *Pertusaria* with mean ascospore length and width from published ranges.

Pertusaria species	Substrate[1]	Spores in ascus	Source for ranges	Length range (μm)	Width range (μm)	Mean[2] length (μm)	Mean[2] width (μm)
P. allanii Zahlbr.	S	2	Galloway (2007)	(150–)175–210(–225)	(50–)60–75(–87)	191.70	67.08
P. celata A.W.Archer & Elix	C	8	Galloway (2007)	90–100(–120)	30–40(–50)	94.87	34.64
P. crassilabra Müll.Arg.	S	8	Kantvilas (2018)[3]			58.4	31.8
P. leucodes C.Knight	C	8	Galloway (1985)	55–70	22.1–27.2	62.05	24.52
P. leucoplaca Müll.Arg.	C	8	Galloway (2007)	70–90(–100)	25–35	79.37	29.58
P. micropora Kremp.	C	8	Galloway (2007)	70–100	25–40	83.67	31.62
P. murrayi Elix & A.W.Archer	C	2	Galloway (2007)	(125–)130–162	50–65	145.12	57.01
P. paratropa Müll.Arg.	S	8	Galloway (2007)	37–55	20–30	45.1	24.5
P. perrimosa Nyl.	S	8	Galloway (1985)	100–122	43–61	110.4	51.21
P. petrophyes C.Knight	S	8	Galloway (2007)	60–85	25–45	71.4	33.5
P. scottii Elix & A.W.Archer	C + M	8	Galloway (2007)	45–55	25–30	49.75	27.39
P. subisidiosa A.W.Archer	C	4	Galloway (2007)	80–95	30–35	87.18	32.4
P. tyloplaca Nyl.	M	8	Galloway (2007)	80–120	30–40	97.98	34.64
P. vallicola Elix & Malcolm	C	2	Galloway (2007)	125–137	50–62	130.86	55.68
P. xanthoplaca Müll.Arg.	S	8	Galloway (2007)	50–75(–90)	25–37	61.2	30.4

1 C = corticolous, C + M = corticolous and muscicolous, M = muscicolous, S = saxicolous.
2 Means calculated as the exponential of the average natural logarithm of range.
3 Means given by Kantvilas (2018) are based on 45 ascospores.

from 17 species to capture mature ascospore morphology. Outlines of photographed ascospores were digitised using Vextractor version 7.2 (VextraSoft 2017) to produce between 30 and 90 points for each outline. Digitising was sequential around an outline, starting and ending at an apex. Outlines were each interpolated to being 50 evenly spaced points based on arc length (Ramsay & Silverman 2002).

To examine ascospore shape, an ellipse was fitted to each 50-point outline using nonlinear least squares (Sampson 1982). If the resulting goodness-of-fit measure (R2) exceeded 99%, and the average residual error was small (i.e., single-digit μm), then the outline was deemed an ellipse. An ellipse about a given origin is completely described by its length and width (Lockwood 1961).

The method of Bartlett (1947) was used to examine whether ascospore measurements require transformation to satisfy statistical assumption of stable variation throughout the measurement range. In other words, what change (if any) is needed to ascospore length and width so that large-spored species have similar variation to the small-spored species?

Summary statistics were estimated by resistant methods (i.e., resisting the undue influence of any extreme or outlier ascospore measurements): the median to estimate the mean, and the median absolute deviation (MAD) to estimate the standard deviation (Venables & Ripley 2002). In other words, the median is the measure of centre or average, and the MAD the measure of spread in the measurements, referred to as the mean and standard deviation respectively.

Covariance between ascospore length and width was estimated using the similarly resistant cov.rob function of the R package MASS with default argument values (Venables & Ripley 2002). The 95% confidence region of mean ascospore length and width for each species was estimated by a multiplier of the pooled covariance of all species. The value of the multiplier was the square root of the chi-square 0.95 quantile on 2 degrees of freedom (x_2^2), divided by the number of ascospores measured for a species (Mardia et al. 1992).

Mature ascospores were not available for 15 of the known verrucoid Aotearoa / New Zealand species (Table 2). Mean ascospore length and width were estimated using the centre of the published ranges that excluded extreme values (Table 2). Confidence regions for these 15 species used the pooled covariance of the 23 measured species and assumed that published ranges were based on measuring at least ten ascospores.

All calculations, analyses and graphics used the open-source R software, version 4.41 (R Core Team 2024). Confidence regions were drawn using the ellipse function of the car package (versions 3.1–3, Fox & Weisberg 2019).

Results

Ellipses fitted to the 69 ascospore outlines all had goodness of fit, R^2, that exceeded 99% (minimum 99.07%, maximum 99.9%, median 99.87%). Residual standard deviation ranged from 0.14 to 4.3 µm with a median of 0.98 µm. That is, of the observed distance variation from the centre of an ascospore to the outer wall, more than 99% was explained by a fitted ellipse to within approximately ± 2 µm. Examples of the fitting are shown in Figure 1, and a summary of all 69 ascospores is given in Supplementary Table S1.

The close approximation of an ellipse to each outline justified proceeding with length and width as being complete descriptors for both ascospore size and shape.

Species standard deviation of ascospore length had a significant linear relationship with mean length ($p \ll 0.01$). In other words, for each species, the standard deviation was expected to be a constant proportion (15%) of ascospore length. Following Bartlett (1947) (per equation 3), all subsequent calculations were based on a natural logarithmic scale for ascospore measurements. Summary statistics were subsequently returned to a micron scale through the exponential function.

Confidence regions for each species are displayed on logarithmic scales, where smaller regions denote those species with more ascospores measured (Figures 2, 3A, 3B). Many species were clearly distinct from each other on mean ascospore length and width, which was enhanced when including substrate and ascus character. For example, while *Pertusaria alboatra* and *P. graphica* have similar ascospore width, and *P. alboatra* has a mean 10 µm longer ascospore, their difference becomes more notable when comparing substrate (corticolous vs. saxicolous) and ascus (two-spored vs. four-spored). On the other hand, of the larger ascospore-measured species, only two pairs (*P. southlandica / P. thiospoda* and *P. allosorodes / P. sorodes*) clearly overlap in terms of mean size, shape, substrate and numbers of spores in ascus (Figure 2). The latter two species also overlap *P. allanii*, which is distinguished by its saxicolous substrate (Table 2).

Overlap and separation are more complex among the eight-spored-ascus species. For easier visual assessment, these 23 species were separated by substrate into saxicolous and non-saxicolous (Figures 3A and 3B respectively). Of the eight saxicolous species, only three (*P. crassilabra*, *P. hypoxantha* and *P. xanthoplaca*) clearly overlap (Figure 3A). The other five saxicolous species show little or no overlap in mean ascospore length and width.

The 15 non-saxicolous, eight-spored-ascus species appear to separate into four, possibly five groups (Figure 3B). While groups of species suggest regions that don't overlap, within the groups there are clearly overlapping confidence regions for species unlikely to differ on mean ascospore length and width. Of the measured species, such overlap happened for two pairs of species: *P. albissima / P. bartlettii* and *P. erumpescens / P. parvula* (Figure 3B). Therefore, six species with mean ascospore lengths between 70 and 110 µm have the most overlap in possibly two groups each with three species.

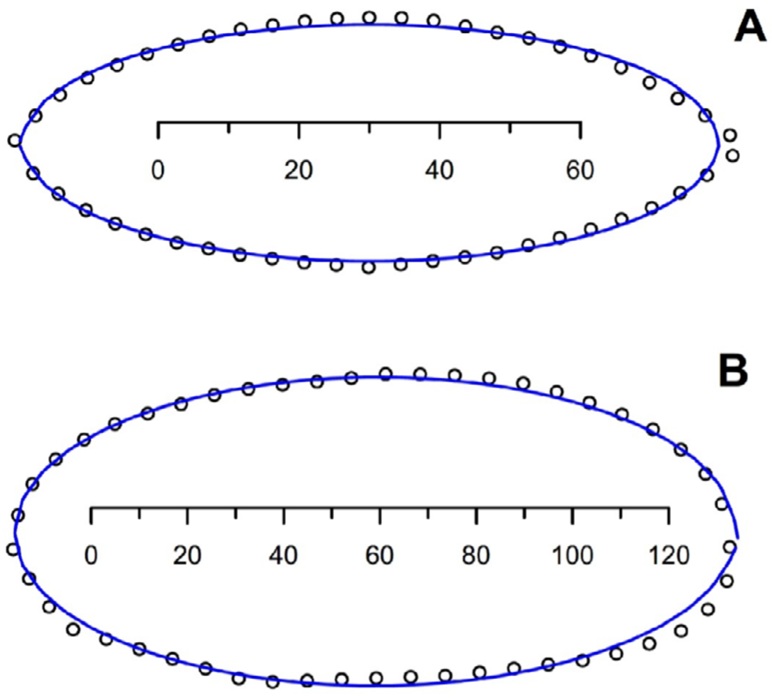


Figure 1. Examples of ellipses (lines) fitted to ascospore outline points (open circles), scale bar in μm. **A.** *Pertusaria subplanaica*. **B.** *P. knightiana*.

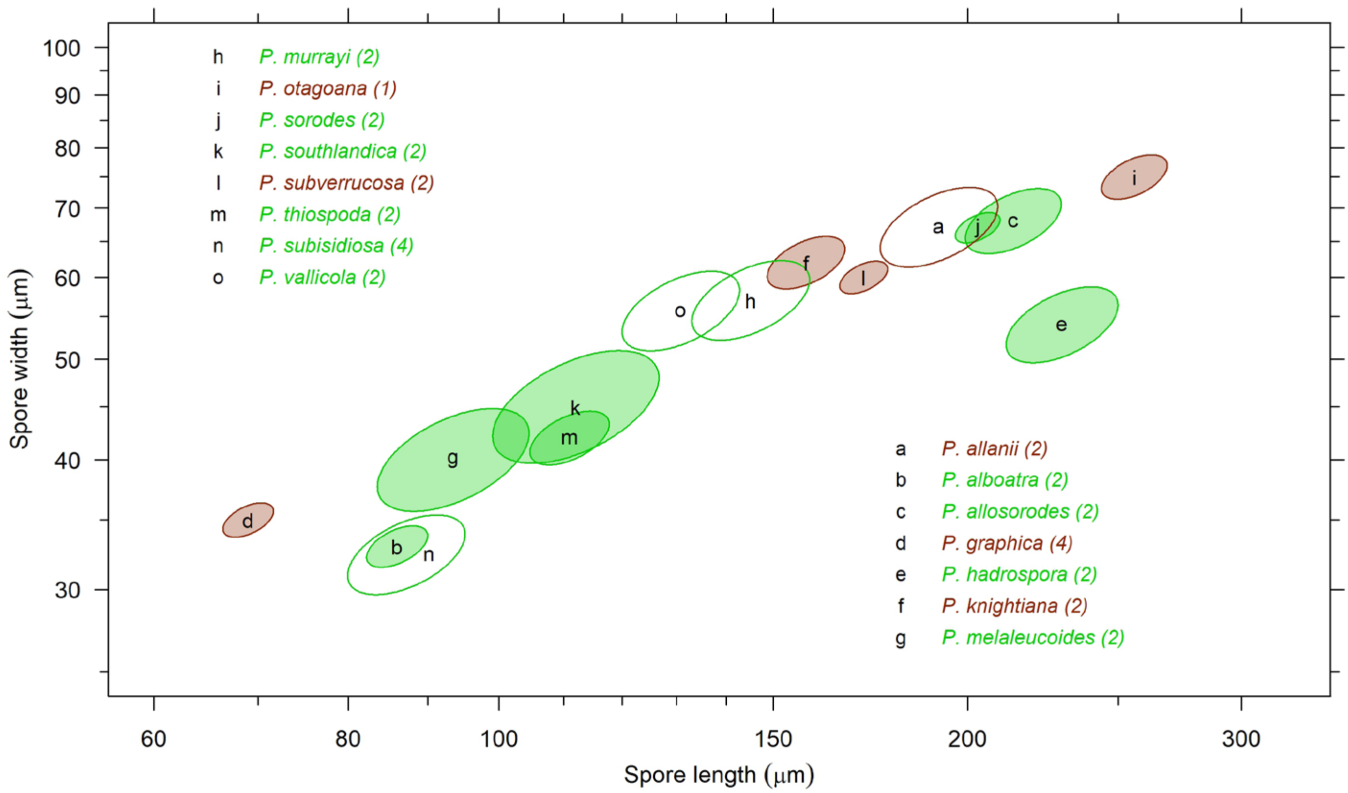


Figure 2. Mean ascospore length and width and a surrounding 95% confidence region for species with one, two or four ascospores in their asci. Dark-orange shading denotes saxicolous species, and green shading the non-saxicolous species. Unshaded regions represent species with means that were estimated only from published ranges in Table 2. In parentheses are the number of ascospores in the ascus for each species.

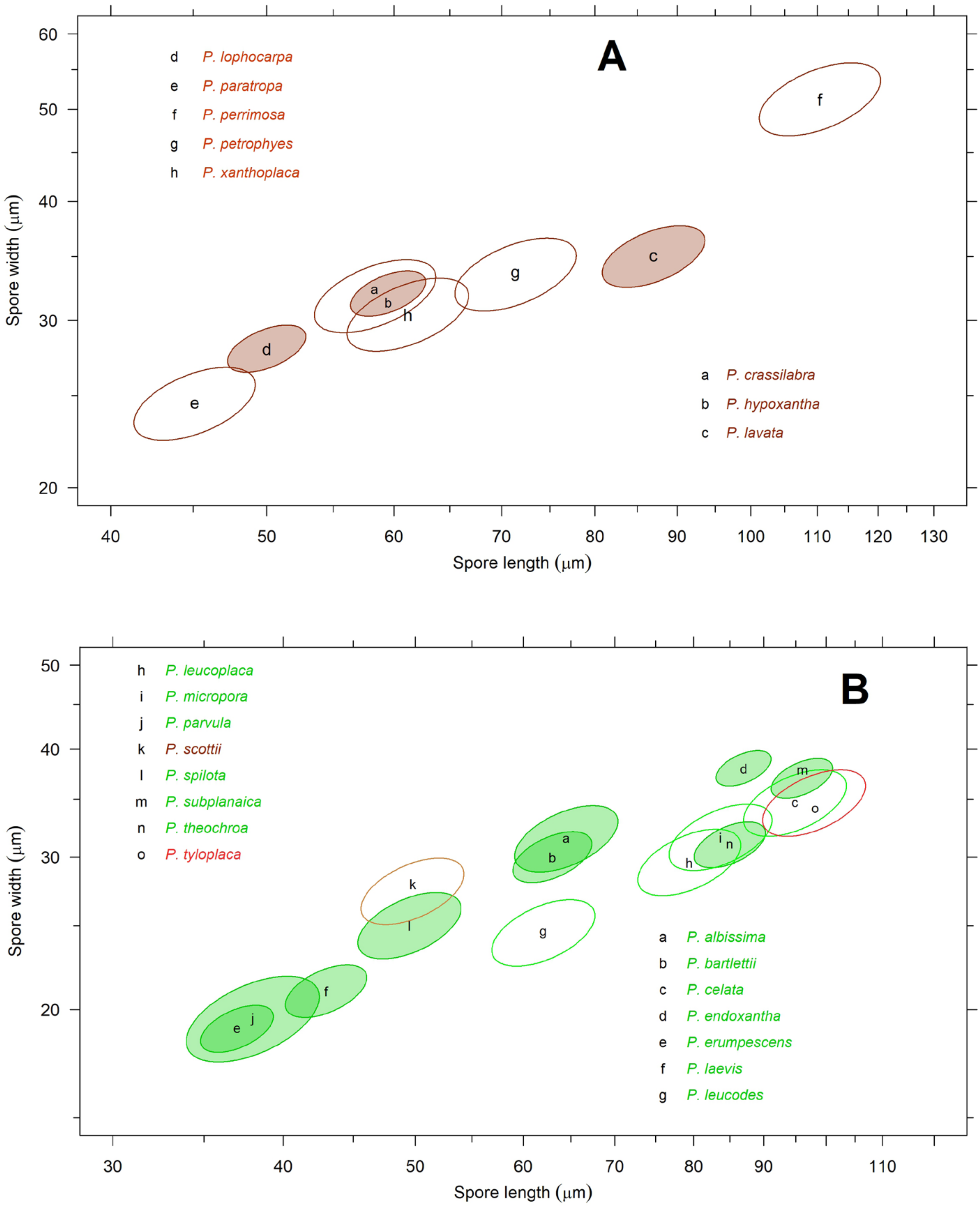


Figure 3. Mean ascospore length and width and a surrounding 95% confidence region for species with eight ascospores in their asci. Unshaded regions represent species with means that were estimated only from published ranges in Table 2. **A.** Saxicolous species. **B.** Non-saxicolous species with eight-spore asci (green denotes corticolous, brown for species found on plant debris or moss substrate).

Discussion

Our results demonstrate that graphical methods could serve as a primary tool for identifying verrucoid *Pertusaria* taxa in Aotearoa / New Zealand, sometimes to a single species. Averaged ascospore measurements of any unknown verrucoid *Pertusaria* can be located in Figures 2 and 3 to identify the nearest species, or at least a small group of candidate species.

Unlike a fixed experiment design, our sampling utilised all available specimens and mature ascospores that were to hand. This study provides strong support for treating *Pertusaria* ascospores as exact ellipsoids in three-dimensional space. All digitised outlines conform to an elliptical shape with deviations often being single-digit microns in magnitude without any consistent outline harmonics, or patterns that could separate species. Because an ellipse is completely described by its length and width, any additional ascospore outline measurements are superfluous when describing shape.

Taxonomic descriptions of ascospore shape have used terms such as 'broadly', 'pointed', 'fusiform', 'subfusiform' or 'ends rounded' to describe the apices (Galloway 1985, 2007). The near-perfect elliptical shape of *Pertusaria* ascospores casts doubt on relying on such epithets to describe overall shape of apices, as doing so could introduce unnecessary subjectivity or imprecision into species descriptions.

While all *Pertusaria* ascospores from Aotearoa / New Zealand examined here were elliptical, this is unlikely so for other crustose lichens, for example, *Lecidea* Ach. and *Bacidia* De Not., where ascospores can have curved sides or extremely pointed ends. For such non-elliptical ascospores, the Fourier harmonic analysis methods of Ordynets et al. (2021) may be more effective to describe shape.

Most published ascospore measurements report extremes in parentheses, separately from their estimated range statistics, and are usually based on ten or more ascospores (e.g., Vondrák et al. 2013). This study has a range of sample sizes for species (Table 1), each with an unknown chance of occasional extreme values that might bias or inflate the estimated means and standard deviations. The use of resistant estimators side-steps the need to check each species for extreme or outlying measurements while still providing reliable values for means, standard deviations and covariance.

The order of magnitude range in *Pertusaria* ascospore length being from 30 µm to 300 µm cautions against blind acceptance of the assumption that species with small ascospores can be statistically compared with species with large ascospores. The significant linear relationship between species standard deviation and mean ascospore length confirms a heteroscedasticity that requires a logarithmic transformation to satisfy the assumption of similar variation throughout the measurement range. Such a transformation may not be needed when the ascospore range is smaller, such as when similar species are being compared (e.g., Fogel 1992).

This study broadly supports and utilises the long-known inverse relationship between ascospores in an ascus and ascospore size (e.g., Martin & Child 1972). Here, the largest ascospore lengths and widths are all from one to two ascospore ascii (Figure 2), and the smallest from eight-ascospore ascii (Figures 3A and 3B).

Mean ascospore length and width plus an associated 95% confidence region show the expected range for each individual *Pertusaria* species. Reference to the non-overlapping confidence regions suggests which species are significantly different from each other, and vice versa.

Many species in our study exceeded the five ascospores recomended as a minimum for a specimen description (Vondrák et al. 2013). While our confidence regions are statistically robust, they may appear overly optimistic when applied to specimens where only a few measurements are available. As an example, consider the two saxicolous species *Pertusaria knightiana* and *P. subverrucosa*, with non-overlapping confidence regions (Figure 2). *P. subverrucosa*'s location and 95% confidence region is based on three times the ascospores of *P. knightiana* (Table 1). A 95% confidence region for the mean length and width based on fewer ascospores would be larger for both species, leading to an overlap. As a conservative aid to identifying a species based on the mean of an unknown specimen, confidence regions were recalculated to be the same for all species. The recalculated graphs are presented as Supplementary Figures S1 and S2 for future application to the means of ten ascospores from any undetermined specimen. Supplementary code (HTML and Javascript) is available from the lead author as an alternative to using the graphical methods above.

Our statistical methods prioritise robustness over theoretical efficiency by employing resistant estimators. While guarding against outlier influence, our methods may sacrifice accuracy if measurements do follow a normal or bell-shaped distribution (Venables & Ripley 2002). Future research should explore alternative statistical methods to better understand patterns in measurement variation and investigate possible causes of ascospore size diversity within *Pertusaria.*

In summary, the graphs presented here serve as a quick way to make a preliminary identification of Aotearoa / New Zealand verrucoid *Pertusaria* specimens, requiring only a standard microscope fitted with an eyepiece graticule for measurements. When a specimen's mean ascospore length and width are plotted on a graph referenced to specimen substrate and number of ascospores in the ascus, the number of possible species is substantially reduced. Confirmation of identification requires the comparison of chemistry and thallus features (bearing in mind their possible variability) with published descriptions.

Acknowledgements

Dr Paul D. Sampson (University of Washington) for code to fit ellipses. Dr Allison Knight (Research Associate, University of Otago) and Marley Ford (PhD student at the University of Auckland) for sending lichen material for identification. Dr Colin H. Aldridge (Ōtepoti / Dunedin) for his reading and commenting on our first draft. Dr Alan Fryday (Michigan State University) for his extensive review, comments and references that substantially improved a draft of this article. Professor David Orlovich (Otago University) for his thoughts on meiosis in fungi. The editors and the two anonymous reviewers for their helpful comments and attention to required corrections.

Data Accessibility Statement

No additional spreadsheet or code.

Author Contributions

Anthony (Tony) E. Aldridge: Investigation (equal), visualisation (lead), writing (equal).
Jennifer M. Bannister: Conceptulisation (lead), measurements (lead), data collection (lead), supervision (lead), writing (equal).

References

Archer, A. W. (2012). *Pertusaria* (lichenised Ascomycota) in Australia. *Australian biological resources study*. Available online: http://www.anbg.gov.au/abrs/lichenlist/PERTUSARIA.html [Accessed: 2 September 2025].

Archer, A. W., Elix, J. A. (2013). New species of *Pertusaria* (Pertusariaceae) from Australia and New Zealand. *Telopea*, 15: 111–117. Available online: https://openjournals.test.library.sydney.edu.au/TEL/article/view/7130/7763 [Accessed: 2 September 2025].

Archer, A. W., Elix, J. A. (2018). *A preliminary world-wide key to the lichen genus* Pertusaria *(including* Lepra *species)*. Available online: https://docslib.org/doc/1338435/1-a-preliminary-world-wide-key-to-the-lichen-genus-pertusaria-including [Accessed: 2 September 2025].

Bartlett, M. S. (1947). The use of transformations. *Biometrics*, 3(1): 39–52. https://doi.org/10.2307/3001536

Elix, J. A., Archer, A. W. (2007). Four new species of *Pertusaria* (lichenized Ascomycota) from Australasia. *Australasian Lichenology*, 60: 20–21. https://www.anbg.gov.au/abrs/lichenlist/AL_60.pdf

Er, O. A. H., Reynolds, C. L., Blanchon, D. J. (2015). Additional lichen records from New Zealand 49. *Pertusaria puffina* A.W. Archer & Elix. *Australasian Lichenology*, 77: 28–31. https://www.researchbank.ac.nz/server/api/core/bitstreams/97035a96-0d84-4dde-a298-c6644cc75272/content

Erbisch, F. H. (1969). Ascus and ascospore development of five species of the lichen-forming genus *Pertusaria*. *The Bryologist*, 72: 178–199.

Fogel, R. (1992). Utility of spore length/width ratio in separating *Geopora cooperi* form *cooperi* and *G. cooperi f. gilkeyae*. *Mycologia*, 84(1): 124–127. https://doi.org/10.1080/00275514.1992.12026115

Fox, J., Weisberg, S. (2019). *An R companion to applied regression*. Third edition. Thousand Oaks: Sage. 608 pp.

Fryday, A. F. (2008). Three new species of lichenised fungi with cephalodia from the southern New Zealand shelf islands (Campbell Plateau). *The Lichenologist*, 40(4): 283–294. https://doi.org/10.1017/S002428290800769X

Galloway, D. J. (1985). *Flora of New Zealand lichens*. Wellington: P. D. Hasselberg. 662 pp.

Galloway, D. J. (2007). *Flora of New Zealand lichens: Revised second edition*. Lincoln: Manaaki Whenua Press. 2261 pp.

Honegger, R., Scherrer, S. (2008). Sexual reproduction in lichen-forming ascomycetes. In: Nash, T. H. (ed.), *Lichen biology*. Cambridge: Cambridge University Press. 486 pp. https://doi.org/10.1017/CBO9780511790478

Kantvilas, G. (2018). *Pertusaria crassilabra* Müll.Arg. – A reinstated name for an Australian lichen. *Swainsonia*, 31: 45–48.

Knight, A., Elix, J. A., Archer, A. W. (2011). A new species of *Pertusaria* (lichenized *Ascomycota*, *Pertusariaceae*) from New Zealand. *Australasian Lichenology*, 69: 33–35. https://www.anbg.gov.au/abrs/lichenlist/AL_69.pdf

Lockwood, E. H., (1961). *A book of curves*. Cambridge: Cambridge University Press. 199 pp.

Ludwig, L. R., Kantvilas, G. (2015). Four Tasmanian lichens new to New Zealand. *Australasian Lichenology*, 77: 18–27. https://www.anbg.gov.au/abrs/lichenlist/AL77.pdf

Mardia, K. V., Kent, J., Bibby, J. M. (1992). *Multivariate analysis*. London: Academic Press. 518 pp.

Marshall, A. J., Blanchon, D. J. (2017). Additional lichen records from New Zealand 50. *Pertusaria endoxantha* Vain. *Australasian Lichenology*, 80: 58–60. https://www.researchbank.ac.nz/server/api/core/bitstreams/2a2cccf9-3f0f-46af-8d7d-97a9c93a11a0/content

Martin, W. M., Child, J. (1972). *Lichens of New Zealand*. Wellington: A. H. & A. W. Reed. 193 pp.

Ordynets, A., Keßler, S., Langer, E. (2021). Geometric morphometric analysis of spore shapes improves identification of fungi. *PLOS One*, 16(8): e0250477. https://doi.org/10.1371/journal.pone.0250477

Pyatt, F. B. (1973). Lichen propagules. In: Ahmadjian, V., Hale, M. E. (eds), *The lichens*, pp. 117–143. New York and London: Academic Press. 697 pp.

R Core Team (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available online: https://www.R-project.org/ [Accessed: 2 September 2025].

Ramsay, J. O., Silverman, B. W. (2002). *Applied functional data analysis: Methods and analysis*. New York: Springer. 190 pp.

Sampson, P. D. (1982). Fitting conic sections to “very scattered” data: An iterative refinement of the bookstein algorithm. *Computer Graphics and Image Processing*, 18: 97–108. https://doi.org/10.1016/0146-664X(82)90101-0

Schmitt, I., Fankhauser, J. D., Sweeney, K., Spribille, T., Kalb, K., Lumbsch, H. T. (2010). Gyalectoid *Pertusaria* species form a sister-clade to *Coccotrema* (Ostropomycetidae, Ascomycota) and comprise the new lichen genus *Gyalectaria*. *Mycology*, 1(1): 75–83. https://doi.org/10.1080/21501201003631540

Schmitt, I., Otte, J., Parnmen S., Sadowska-Des, D., Lucking, R., Lumbsch, H. T. (2012). A new circumscription of the genus *Varicellaria* (Pertusariales, Ascomycota). *MycoKeys*, 4: 23–36. https://doi.org/10.3897/mycokeys.4.3545

Sweetwood, G., Lucking, R., Nelsen, M. P., Aptroot, A. (2012). Ascospore ontogeny and discharge in megalosporous Trypetheliaceae and Graphidaceae (Ascomycota: Dothideomycetes and Lecanoromycetes) suggest phylogenetic relationships and ecological constraints. *The Lichenologist*, 44(2): 277–296. https://doi.org/10.1017/S0024282911000740

Venables, W. N., Ripley, B. D. (2002). *Modern applied statistics with S*. Fourth edition. New York: Springer. 495 pp. https://doi.org/10.1007/978-0-387-21706-2

VextraSoft (2017). Vextractor. Available online: http://www.vextrasoft.com [Accessed: 2 September 2025]

Vondrák, J., Frolov, I., Arup, U., Khodosovtsev, A. (2013). Methods for phenotypic evaluation of crustose lichens with emphasis on Teloschistacae. *Чорноморський ботанічний журнал*, 9(3): 382–405. (In English). http://www.irbis-nbuv.gov.ua/cgi-bin/irbis_nbuv/cgiirbis_64.exe?C21COM=2&I21DBN=UJRN&P21DBN=UJRN&IMAGE_FILE_DOWNLOAD=1&Image_file_name=PDF/Chbj_2013_9_3_8.pdf

Wei, X., Schmitt, I., Hodkinson, B., Flakus, A., Kukwa, M., Divakar, P. K., Kirika, P. M., Otte, J., Meiser, A., Lumbsch, H. T. (2017). Circumscription of the genus *Lepra*, a recently resurrected genus to accommodate the “*Variolaria*”-group of *Pertusaria* sensu lato (Pertusariales, Ascomycota). *PLOS One*, 12(7): 8–14. https://doi.org/10.1371/journal.pone.0180284

Table S1. Summary of spore outline statistics and ellipse fitting.

Species[1]	spore	length	width	area	perim	ecc	rss	R2
P. albissima	1	80.1	34.6	2,117	185	0.91	0.38	99.95
P. albissima	2	73.9	32.7	1,857	172	0.90	0.58	99.86
P. albissima	3	95.5	35.8	2,596	215	0.93	0.44	99.95
P. allanii	1	175.9	72.2	10,285	411	0.91	2.00	99.70
P. allanii	2	158.6	74.3	9,370	380	0.89	1.83	99.71
P. allanii	3	236.3	78.3	14,880	535	0.95	3.23	99.53
P. allanii	4	174.0	73.4	10,305	407	0.91	0.98	99.93
P. allosorodes (2013)	1	214.1	69.2	12,118	480	0.95	2.85	99.55
P. allosorodes (2013)	2	191.8	61.8	9,854	431	0.94	1.53	99.84
P. allosorodes (2013)	3	232.6	73.3	13,733	517	0.95	2.85	99.61
P. allosorodes (2013)	4	215.3	72.1	12,397	487	0.95	4.14	99.07
P. allosorodes (2013)	5	226.7	85.9	16,247	531	0.93	3.12	99.56
P. allosorodes (2013)	6	188.0	69.7	10,561	430	0.93	1.51	99.84
P. bartlettii	1	61.5	33.9	1,622	152	0.84	0.32	99.95
P. bartlettii	2	61.8	31.6	1,486	148	0.86	0.29	99.95
P. bartlettii	3	66.3	23.8	1,216	148	0.94	0.66	99.75
P. graphica	1	80.6	33.9	2,196	186	0.91	1.03	99.63
P. graphica	2	64.4	31.6	1,634	156	0.87	0.35	99.94
P. graphica	3	78.3	40.7	2,461	190	0.86	0.51	99.91
P. graphica	4	82.4	38.9	2,437	194	0.88	0.84	99.77
P. hadrospora	1	231.1	61.1	11,132	499	0.97	0.72	99.97
P. hadrospora	2	251.7	69.8	13,898	546	0.96	3.09	99.58
P. hadrospora	3	253.6	62.8	12,512	543	0.97	1.49	99.90
P. hadrospora	4	194.7	50.2	7,815	421	0.97	0.70	99.96
P. hypoxantha (2017)	1	56.3	34.6	1,467	141	0.79	0.30	99.95
P. hypoxantha (2017)	2	51.7	30.3	1,206	129	0.81	0.26	99.95
P. hypoxantha (2017)	3	64.7	32.4	1,612	154	0.87	0.25	99.97
P. hypoxantha (2017)	4	80.4	35.0	2,114	185	0.91	0.78	99.78
P. knightiana	1	148.9	63.9	7,605	350	0.91	1.12	99.87
P. knightiana	2	155.4	72.6	9,082	372	0.88	0.87	99.93
P. knightiana	3	176.5	68.7	9,920	409	0.92	1.97	99.71
P. knightiana	4	164.9	64.3	8,707	383	0.92	1.01	99.91
P. lophocarpa	1	63.2	32.8	1,631	153	0.85	0.25	99.97
P. lophocarpa	2	49.1	25.6	995	121	0.85	0.16	99.98
P. lophocarpa	3	51.8	29.3	1,168	128	0.83	0.19	99.97
P. melaleucoides	1	95.9	41.0	3,046	222	0.90	0.37	99.96

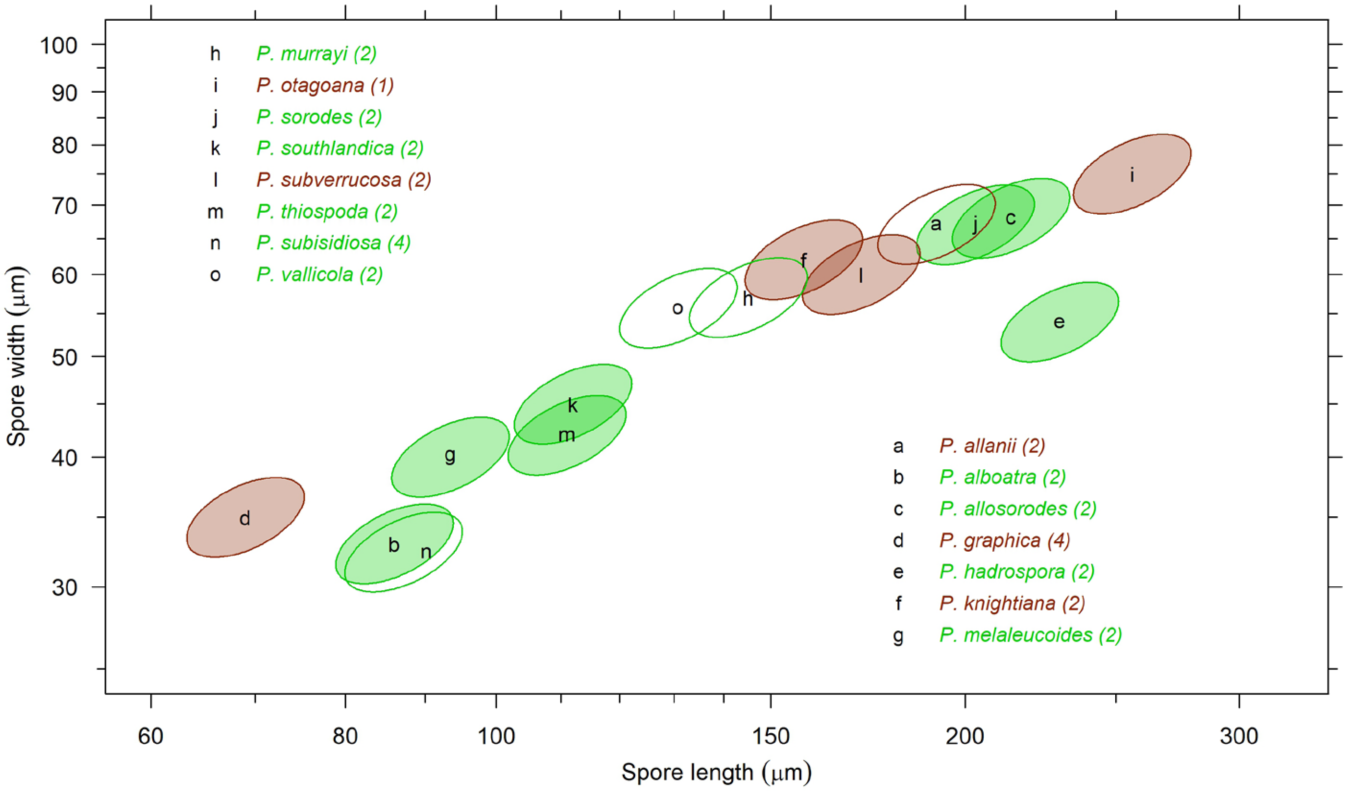
P. melaleucoides	2	95.8	38.8	2,982	223	0.91	0.70	99.88
P. melaleucoides	3	108.8	47.0	4,024	255	0.90	0.73	99.90
P. otagoana	1	265.0	89.2	19,498	605	0.94	4.43	99.31
P. otagoana	2	333.3	99.6	27,139	708	0.96	4.29	99.54
P. otagoana	3	247.5	89.1	17,834	562	0.93	1.34	99.93
P. otagoana	4	246.6	71.7	14,972	552	0.96	2.46	99.74
P. parvula	1	34.8	19.4	544	87	0.82	0.15	99.97
P. parvula	2	32.6	17.5	464	81	0.84	0.27	99.86
P. parvula	3	64.1	36.6	1,907	164	0.82	0.68	99.79
P. parvula	4	36.7	18.2	566	91	0.86	0.40	99.76
P. sorodes	1	173.7	66.4	9,224	397	0.92	1.04	99.91
P. sorodes	2	221.8	76.9	13,803	504	0.94	1.72	99.85
P. sorodes	3	200.7	80.1	13,127	468	0.91	1.50	99.87
P. sorodes	4	212.3	86.2	15,174	500	0.91	1.83	99.83
P. sorodes	5	218.9	68.1	12,056	487	0.95	2.79	99.58
P. sorodes	6	224.9	71.4	12,865	502	0.95	2.57	99.66
P. sorodes	7	135.9	54.6	6,006	317	0.92	1.74	99.62
P. sorodes	8	191.9	79.3	12,324	450	0.91	1.35	99.89
P. sorodes	9	167.2	77.0	10,352	400	0.89	1.15	99.90
P. subplanica	1	102.0	35.5	2,619	223	0.94	0.70	99.87
P. subplanica	2	84.3	45.2	2,863	204	0.85	0.46	99.94
P .subplanica	3	89.4	43.7	2,996	212	0.87	0.58	99.91
P. subplanica	4	97.7	34.0	2,461	214	0.94	0.54	99.92
P. subplanica	5	85.8	31.4	2,108	194	0.93	0.38	99.95
P. subverrucosa	1	187.9	71.8	10,585	427	0.92	0.98	99.93
P. subverrucosa	2	203.5	64.8	10,817	455	0.95	1.61	99.84
P. subverrucosa	3	209.9	66.6	11,358	470	0.95	1.73	99.83
P. subverrucosa	4	220.1	100.5	17,958	527	0.89	1.98	99.83
P. subverrucosa	5	197.4	93.8	14,493	470	0.88	1.38	99.89
P. thiospoda	1	123.2	47.3	4,907	290	0.92	1.46	99.68
P. thiospoda	2	123.6	42.9	4,596	285	0.93	1.24	99.76
P. thiospoda	3	123.7	47.7	4,928	288	0.92	0.98	99.86
P. thiospoda	4	105.4	42.3	3,674	248	0.92	1.61	99.47
P. thiospoda	5	113.0	42.8	4,051	263	0.92	0.81	99.88

1 Species with year were published after those in Galloway (2007).

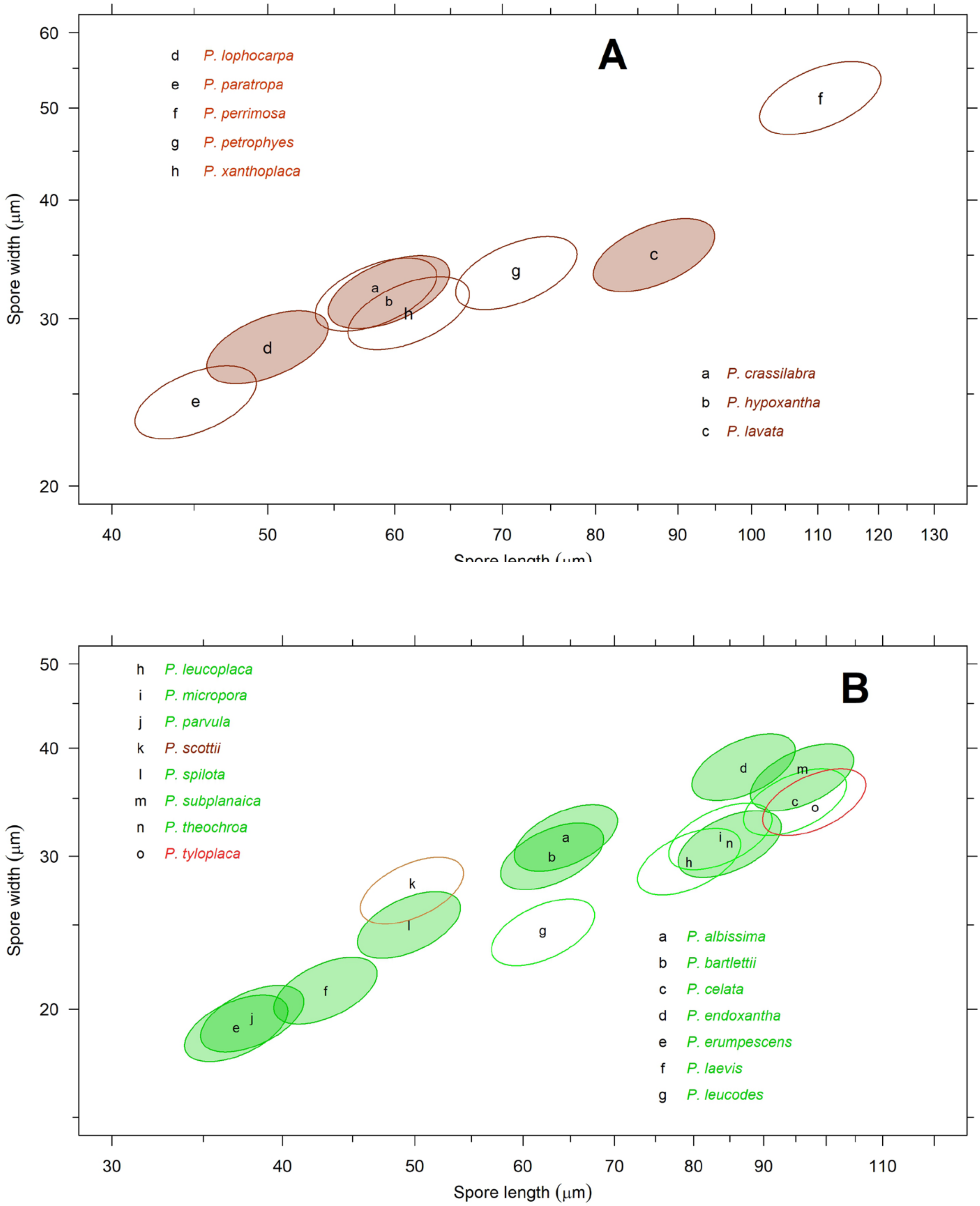
length = spore length (μm)
width = spore width (μm)
area = spore area (μm^2)
perim = perimeter (μm)

ecc = elliptical eccentricity sqrt(1 - ((width/2)^2 / (length/2)^2))
rss = residual standard deviation from elliptical fit (μm)
R2 is goodness of elliptical fit (%)

Highlighted rows relate to spores in Figure 1, *Petusaria knightiana* (spore 1) and *P. subplanica* (spore 1).



Supplementary Figure S1. Mean length and width and a surrounding 95% confidence region (ten ascospores) for species with one, two or four ascospores in their asci. Dark-orange shading denotes saxicolous species, and green shading the non-saxicolous species. Unshaded regions represent species with means that were estimated only from published ranges in Table 2. In parentheses are the number of ascospores in the ascus for each species from Table 1.



Supplementary Figure S2. Mean length and width and a surrounding 95% confidence region (ten ascospores) for species with eight ascospores in their asci. Unshaded regions represent species with means that were estimated only from published ranges in Table 2. **A.** Saxicolous species. **B.** Non-saxicolous species with eight-spore asci (green denotes corticolous, brown for species found on plant debris or moss substrate).