RESEARCH ARTICLE

The cup fungus *Pestalopezia brunneopruinosa* is *Pestalotiopsis gibbosa* and belongs to Sordariomycetes

**Kyoko Watanabe**1*, Shunsuke Nozawa1, Tom Hsiang2, Brenda Callan3

1 Graduate School of Agriculture, Tamagawa University, Machida, Tokyo, Japan, 2 Environmental Sciences, University of Guelph, Guelph, Ontario, Canada, 3 Pacific Forestry Centre, Natural Resources Canada, Victoria, British Columbia, Canada

* wkyoko@agr.tamagawa.ac.jp

**Abstract**

*Pestalopezia brunneopruinosa*, the type species of *Pestalopezia* in Leotiomycetes, produces typical cup-shaped ascomata. Because its asexual morph has conidia comprised of five cells including apical and basal appendages and three pigmented median cells, it was first described as *Pestalotia gibbosa*, which belongs to Sordariomycetes. This contradiction has not been resolved due to the difficulty in isolating this fungus in culture. In this study, we isolated separate strains from the sexual morph and the asexual morph for molecular analysis. Phylogenetic trees of Sporocadaceae based on internal transcribed spacer, partial β-tubulin, and partial translation elongation factor 1-alpha sequence datasets revealed that both strains fall into the same taxon, in a clade in *Pestalotiopsis* sensu stricto alongside *P. gaultheriae* and *P. spathulata*. We provide the first evidence that fungi producing cup-shaped ascomata in *Pestalotiopsis* belong to Sordariomycetes, and we have proposed the transfer of *Pestalopezia brunneopruinosa* to *Pestalotiopsis gibbosa*.

**Introduction**

*Pestalopezia brunneopruinosa* (Zeller) Seaver is a leaf spot pathogen on salal (*Gaultheria shallon* Pursh) that produces asci on an apothecium as a sexual morph [1]. The asexual morph of *Pestalopezia brunneopruinosa* resembles that of *Pestalotiopsis* sensu lato (s. lat.) and was first described independently by Harkness [2] as *Pestalotia gibbosa*. Thus, it has been suspected that *Pestalopezia brunneopruinosa* and *Pestalotia gibbosa* are the same fungus, because the two fungi have been found in close proximity on the same leaves. Bonar [3] demonstrated that cultures from germinated ascospores of *Pestalopezia brunneopruinosa* produced conidia that were the same as that of *Pestalotia gibbosa*. Seaver [4] likewise concluded that *Pestalopezia brunneopruinosa* was the sexual morph of *Pestalotia gibbosa*. However, phylogenetic analyses of both fungi to clarify their relationship has not been previously conducted.

The genus *Pestalotia* was established by De Notaris [5]. Subsequently, Steyaert [6] split the genus *Pestalotia* into *Pestalotia* sensu stricto (s. str.) (conidia composed of 6 cells), *Pestalotiopsis* (5 cells) and *Truncatella* (4 cells), although many species were still retained in *Pestalotia* s.
Recently, *Pestalotiopsis s. lat.* was further split into three genera, *Pestalotiopsis s. st.*, *Neopestalotiopsis*, and *Pseudopestalotiopsis*, based on morphology and molecular phylogeny [7]. These fungi belong to Sporocadaceae within Sordariomycetes [8]. The Harkness description of *Pestalotia gibbosa* conidia (three pigmented median cells in five-celled versicoloured conidia, with septa darker than the rest of the cell), is similar to that of *Neopesotalotis*. However, the current taxonomic position of *Pestalotia gibbosa* is unclear, especially since the disposition of this fungus in Sordariomycetes was made without molecular data support. The sexual morph of *Pestalotiopsis s. lat.* was determined by Barr [9] to be *Pestalosphaeria* which produces three celled-ascospores and perithecial ascocarps. Réblova et al. [10] proposed using the name *Pestalotopsis* rather than *Pestalosphaeria* as the currently accepted name, following recent botanical code changes, but there was no mention of the name *Pestalopezia* in this argument. *Pestalopezia*, *Pestalotiopsis*, and *Pestalosphaeria*, are, however, included in a “without-prejudice list of generic names of fungi for protection under the International Code”[11].

*Pestalopezia brunneopruinosa*, the sexual morph, was classified as a member of the Leotiomycetes [12] because it produces cup-shaped ascomata. Thus, the genus names of the sexual and asexual morphs are currently forced into different taxonomic classes. Beimforde et al. [13] conducted a phylogenetic analysis by combining fossil data and molecular data (18S rDNA, 28S rDNA, RPB1, and RPB2) and showed estimated lineages of both families diverged during the Permian or Carboniferous periods and Leotiomycetes and Sordariomycetes are sister clades. Their results indicate that these families, both of which produce inoperculate asci, are closely related in the molecular phylogenetic tree. However, there is no report that fungi belonging to Sordariomycetes can produce cup-shaped ascomata. The aim of this study was to clarify the taxonomic position of *Pestalopezia brunneopruinosa* with respect to *Pestalotia gibbosa*, and to determine the name for this fungus based on the concept of one fungus, one name [14, 15].

**Materials and methods**

**Sample collection and isolation**

Diseased leaves of salal (Fig 1) were collected from Sandcut Beach trail near Shirley, Vancouver Island BC, Canada in 2013. Several isolates that originated from single conidia in acervuli were cultured from diseased leaves. Isolates were also initiated from ascospores in an ascus, but ejected ascospores failed to individually germinate. Subsequent transfers from the ascus isolate were made from single conidia. Isolates obtained from the asexual morph: NOF 3175/TAPI3K_P3, and from the sexual morph: NOF 3176/TAPI3K_ca_as2 were maintained on PDA (potato dextrose agar, Eiken, Tokyo, Japan) at 15°C, examined to assess taxonomic position, and deposited in The Fungus Culture Collection of the Northern Forestry Centre, Edmonton, Alberta, Canada and Tamagawa University, Machida, Tokyo, Japan. A voucher specimen containing both apothecia and acervuli was deposited in the Pacific Forestry Centre Forest Pathology Herbarium (DAVFP 29689). Information of new combination in Nomenclature was deposited in the Mycobank (http://www.mycobank.org/defaultinfo.aspx?page=Home: MB#824630).

**DNA extraction and molecular analysis**

DNA from each strain was extracted using the Qiagen DNA Mini Kit (Qiagen, Tokyo, Japan) following the manufacturer’s protocol. Internal transcribed spacer (ITS), β-tubulin, and partial translation elongation factor 1-alpha (tefl) gene regions were amplified as described previously.
[16–19], using primers ITS1/ITS4, Bt2d/Bt2c, and pest_ef_f/EF1-1567R, respectively. These primers target regions that are approximately 550 bp, 560 bp, and 530 bp in size, respectively.

To confirm the culture was isolated from the sexual morph, DNA was extracted from a single apothecium from DAVFP 29689 by CTAB [20], and ITS was amplified using our designed primer PES3 (5’-GGCCTACCCTGTAGCGCTT-3’ and ITS4.

Polymerase chain reaction (PCR) products were purified using ExoSAP-IT (GE Healthcare Japan, Tokyo, Japan) and sequenced using the ABI 310 DNA sequencer (ABI, Tokyo, Japan). These sequences have been deposited in the DNA Data Bank of Japan (https://www.ddbj.nig.ac.jp/index-e.html: accession numbers are shown in Table 1).

The results of the preliminary sequence homology search using BLAST were that the two Vancouver Island salal isolates, NOF 3175/TAP13K_P3 and NOF 3176/TAP13K_ca_as2, fell into Pestalotiopsis s. str. Additional sequence data for phylogenetic analysis were obtained from 7 other previously unpublished strains (listed in bold in Table 1), and 43 other strains published in previous studies [7, 21]. To generate phylogenies based on ITS, β-tubulin, and tef1 sequences, Seiridium spp., members of Amphipharciaceae (outgroup) and Phlogicylin-driaceae, were chosen because they are phylogenetically close to Sporocadaceae. The dataset of each genomic region (ITS, β-tubulin, and tef1) was aligned using MAFFT [22]. All positions containing gaps and missing data were deleted from the analysis. The strength of internal branches from the resulting tree was tested using the bootstrap analysis [23] with 1,000 replications.

Sequence data comprising the aligned dataset were subjected to maximum-likelihood (ML), neighbor-joining (NJ) and maximum-parsimony (MP) phylogenetic analyses using MEGA software Version 7 [24]. Molecular analyses using the ML method were performed using HKY+G+I nucleotide substitution model for ITS, β-tubulin, and tef1. Initial trees for the heuristic searches were automatically generated by applying the NJ and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with a higher log-likelihood value. Evolutionary history was inferred using the NJ method [25]. The tree was drawn to scale with branch-length units equivalent to those of the evolutionary distances used to infer phylogeny. Evolutionary distances were computed using the Kimura 2-parameter method [26] as the number of base substitutions per site. MP trees were generated using the tree-bisection-regrafting (TBR) algorithm and search level 3, which generates initial trees by randomly adding sequences (10
Table 1. Source of species for molecular analyses and the DNA database accession number.

| Species                  | Culture No. | Location          | Host                          | GenBank accession     |
|--------------------------|-------------|-------------------|-------------------------------|-----------------------|
|                          |             |                   |                               |                       |
| Pestalotiopsis gibbosa   | NOF 3175/TAP13K_P3 | Canada            | Gaultheria shallon            | LC311589 LC311590 LC311591 |
| (syn. Pestalotia gibbosa, this study) |             |                   |                               |                       |
| P. gibbosa                | NOF 3176/TAP13K_ca_as2 | Canada            | Gaultheria shallon            | LC311586 LC311587 LC311588 |
| (syn. Pestalopezia brunneoprunosa, this study) |             |                   |                               |                       |
| Pestalotiopsis adusta    | MAFF 240993| Japan              | Cocos nucifera                | LC311592 LC311593 LC311594 |
|                          | CBS 384.65  | USA                | Arceuthobium campylopodum     | LC311595              |
| P. pallidotheae          | CBS 331.92  | Singapore          | Arenga undulatifolia          | LC311596              |
|                          | CBS 272.29  | New Zealand        | Knightia sp.                  | LC311597              |
| P. chamaeops              | CBS 186.71  | Italy              | Chamaeops humilis             | LC311598              |
| P. clavata                | MFLUCC 12–0268 | China            | Buxus sp.                     | LC311599              |
| P. colombiensis           | CBS 118553  | Colombia           | Eucalyptus eurograndis        | LC311600              |
| P. diplocissae            | CBS 256.53  | Australia          | Delonix regia                 | LC311601              |
| P. ericaecarum            | MFLUCC 2439 | Japan              | Delonix regia                 | LC311602              |
| P. furcata                | MFLUCC 12–0054 | Thailand          | Delonix regia                 | LC311603              |
| P. gaultheriae            | IFRD 411–014| China              | Gaultheria forrestii          | LC311604              |
| P. grevillea              | CBS 114127  | Australia          | Grevillea sp.                 | LC311605              |
| P. humus                  | CBS 336.97  | Papua New Guinea   | Coastal soil                  | LC311606              |
| P. kenyana                | CBS 442.67  | Kenya              | Coffea sp.                    | LC311607              |
| P. monochaeta             | CBS 144.97  | Japan              | Cocos nucifera                | LC311608              |
| P. neglecta (this study)  | TAP1100'/MAFF239735 | Japan            | Quercus robur                 | LC311609              |
| P. novae-hollandiae       | CBS 130973  | Australia          | Banksia grandis               | LC311610              |
| P. oryzae                 | CBS 353.69  | Denmark            | Oryza sativa                  | LC311611              |
| P. papuana                | CBS 331.96  | Papua New Guinea   | Coastal soil                  | LC311612              |
| P. parva                  | CBS 265.37  | Japan              | Delonix regia                 | LC311613              |
| P. pallidotheae           | MAFF 240993* | Japan             | Pieris japonica               | LC311614              |
| P. portugalica            | CBS 393.48  | Portugal           | Quercus myrsinaefolia         | LC311615              |
| P. rhododendri            | IFRDCC 2399 | China              | Quercus myrsinaefolia         | LC311616              |
| P. scoparia               | CBS 176.25  | -                  | Chamaecyparis sp.             | LC311617              |
| P. spatulata              | CBS 356.86  | Chile              | Gevuina avellana              | LC311618              |
| P. teleopea               | CBS 114161  | Australia          | Teleopea sp.                  | LC311619              |
| Pestalotiopsis sp.1 (this study) | TAP0K00Kin | Japan             | Osmanthus fragrans var. | LC311620              |
| Pestalotiopsis sp.2 (this study) | TAP0E0SA*  | China              | Camellia sasanqua             | LC311621              |
| Pseudopestalotiopsis cocos | CBS 272.29  | Indonesia          | Cocos nucifera                | LC311622              |
| Ps. theae                 | MFLUCC 12–0055/CPC 20281 | Thailand | Camellia sasanqua             | LC311623              |
| Ps. myanmarina            | NBR 112264* | Myanmar            | Areverha carambola             | LC311624              |
| Ps. viennensis            | NBR 112252* | Vietnam            | Fragaria sp.                  | LC311625              |
| Neopestalotiopsis australis | CBS 114159  | Australia          | Teleopea sp.                  | LC311626              |
| N. cubana                 | CBS 600.96  | Cuba               | Leaf litter                   | LC311627              |
| N. foedans                | CGMCC 3.9123 | China             | Mangrove plant                | LC311628              |
| N. honolulua              | CBS 114495  | USA: Hawaii        | Teleopea sp.                  | LC311629              |
| N. javaensis              | CBS 257.31  | Indonesia: Java    | Cocos nucifera                | LC311630              |

(Continued)
replicates). Consistency, retention, homoplasy, and composition indices were calculated for parsimony-informative sites. The resulting trees were printed using TreeView v. 1.6.6 [27] and, together with the alignments, deposited as S21431 in TreeBASE (https://www.treebase.org/treebase-web/home.html).

### Morphological observations

Morphological observations were made from symptomatic salal leaves collected in 2013 (DAVFP 29689) and from a single dried herbarium specimen DAVFP 11308. The latter was collected in 1959, also from Vancouver Island, and determined as *Pestalopezia brunneopruinos*a by W. Ziller (S1 Fig). The asexual and sexual morphs were observed and measured in water using light microscopy (BX 51, Olympus Tokyo, Japan).

### Nomenclature

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS ONE article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number [urn:lsid:mycobank.org:MycoBank:824630] contained in this publication to the prefix http://www.mycobank.org/MB/. The online version of this work is archived and available from the following digital repositories: PubMed Central, LOCKSS.

### Results

**Phylogenetic analysis**

In addition to the Vancouver Island collections preliminarily identified as *Pestalotia gibbosa* (NOF 3175/TAP13K_P3, Culture from conidia) and *Pestalopezia brunneopruinos*a (NOF...
3176/TAP13K_ca_as2, Culture from ascospores), a total of 52 strains, including Pestalotiopsis (30 strains with two obtained from sexual morphs), Neopestalotiopsis (11 strains), and Pseudo-pestalotiopsis (4 strains including one obtained from the sexual morph), were examined (accession numbers shown in Table 1). The sequence matrix used for phylogenetic analyses contained at least 1258 nucleotide positions for final data set from sequences 550 bp of ITS, 560 bp of \( \beta \)-tubulin, and 530 bp of tef1. In ML method, the highest log-likelihood was -6657.97. The optimal tree generated using the NJ method had a branch-length of 0.665. An MP tree had a length of 909, consistency index of 0.547, retention of 0.87 and composite index of 0.509. Only the ML tree (Fig 2) is shown here, because the ML, NJ, and MP methods generated similar topologies.

*Pestalotia gibbosa* and *Pestalopezia brunneopruinosa* were placed in the same clade with *Pestalotiopsis gaultheriae* (ML/NJ/MP: 100/100/100). *Pestalotiopsis spathulata* was also closely placed to *Pestalotia gibbosa* and *Pestalopezia brunneopruinosa* with highly supported bootstrap values (ML/NJ/MP: 100/100/99). Furthermore, the ITS sequence obtained from *Pestalotia gibbosa* (NOF 3175/TAP13K_P3) and *Pestalopezia brunneopruinosa* (NOF 3176/TAP13K_ca_as2) were the same as the ITS sequence obtained from DNA extracted directly from an apothecium of DAVFP 29689 (epitype specimen) (S2 Fig).

**Morphological comparisons**

Our observations of the apothecia from DAVFP 11308 and 29689 are similar to those of Seaver’s description [4] of *Pestalopezia brunneopruinosa*, with few exceptions. Seaver’s ascospore measurements were slightly larger than the Vancouver Island DAVFP (VI) specimens at 7–10 x 14–20 um, plus we observed in the VI collections that mature ascospores eventually darken to brown rather than remaining hyaline (Fig 3, S3 Fig). We also observed a ring-shaped ascus apparatus in DAVFP 29689 which stained blue in Melzer’s reagent, but only in scattered mature asci. These morphological variations are relatively minor and likely reflective of the state of maturity of Seaver’s material (S1 Table). We also compared our observations and measurements of the conidial states of DAVFP 11308 and 29689 from leaves to published descriptions and specimens of conidia of *Pestalopezia brunneopruinosa*, *Pestalotiopsis gaultheriae*, and *P. spathulata* (Table 2). With the exception that *P. spathulata* has fewer and longer appendages [7], all are morphologically very similar.

**Taxonomy**

*Pestalotiopsis gibbosa* (Harkn.) Kyoko Watan., Nozawa & B. Callan, comb. nov. [urn:lsid:mycobank.org:Mycobank:824630]

= *Pestalotia gibbosa* Harkn. Bull. Calif. Acad. Sci. 2: 439, 1887 MB#191515

= *Dermatea brunneopruinosa* Zeller, Mycologia 26: 291, 1934 MB#259032

= *Pestalopezia brunneopruinosa* (Zeller) Seaver, Mycologia 34: 300, 1942 MB#289174

≡*Pestalotiopsis gaultheriae* Y.M. Zhang, Maharachch. & K.D. Hyde, Sydowia 65: 121, 2013 MB#803236

**Epitype (Fig 3)**

DAVFP 29689, Sandcut Beach trail, Shirley, Vancouver Island BC, Canada, 48.4173°N, 124.0185°W March 5, 2013, on leaves of *G. shallon* Pursh collected by B. Callan and M.
Brannigan. Ex-epitype NOF 3176/TAP13K_ca_as2 was isolated from a conidium transferred from a colony originating from a single ascus.

**Ascocarp**: Apothecium developing on the upper surface of pale tan to light brown necrotic areas of attached living leaves, sessile or with short stalk approximately 0.5–2 mm in diameter, cup-shaped, with a wood brown to yellowish brown furfuraceous exterior.

**Hymenium**: fuscous when immature, becoming black at maturity because of the dark tips of paraphyses forming the epithelium; **asci**: 115–150 μm in length (including a short stalk) × 11–15 μm in diameter (n = 20), eight-spored, unitunicate, cylindrical, with slightly pointed apex, apical apparatus ring-shaped and staining blue in Melzer’s reagent, but only when fully mature; **ascospores**:

---

Fig 2. Maximum-likelihood (ML) tree with length 743 determined by analysis of the combined ITS, β-tubulin, and tef1 sequence matrix. Numbers (ML/NJ/MP) and hyphens on the branches represent the bootstrap values (%) for each node, calculated from 1,000 replicates; only values > 80% are shown. NJ: neighbor-joining, MP: Maximum-parsimony. *: ex-holotype cultures. Blue texts indicate strains producing sexual morphs.

https://doi.org/10.1371/journal.pone.0197025.g002

---
5–8 × 11–16 μm (n = 20), ellipsoidal to ovate, at first hyaline, becoming dark brown when mature, one-seriate; paraphyses: slender and clavate, light brown at their tips in Melzer’s reagent.

Conidiomata: Acervuli erumpent through the upper surface of the leaf epidermis, frequently in a zonate pattern in necrotic lesions. Lesions frequently coalescing, turning the leaf

Table 2. Morphological comparison of asexual morphs of Pestalotiopsis gibbosa and related species.

| Species                          | Three median cells | Apical appendages |
|----------------------------------|--------------------|-------------------|
|                                  | Size (length × width, μm) | Length (μm) | Colour           | Number | Size (length, μm) | Tip        |
| Pestalopezia brunneoprinoso       | 25–30 × 8–10.5      | 16–20            | concolorous, olivaceous | 2–4 (3) | 30–60              | knobbed    |
| Pestalopezia brunneoprinoso (DAVFP 11308) | 22.5–32 × 8–13.5    | 13–20            | versicolorous, dark brown | 1–4 (3) | 20–48              | knobbed    |
| Pestalotiopsis gibbosa (DAVFP 29689) | 24–31 × 7.5–10      | 15.5–22.5        | versicolorous, dark brown | 2–4 (3) | 22–61              | knobbed    |
| Pestalotiopsis gaultheriae        | 23–31 × 7–9.5       | –                | versicolorous, dark brown | 3       | 15–50              | knobbed    |
| Pestalotiopsis spathulata         | 24–32 × 7.5–9.5     | 13–20            | versicolour           | 2–5     | 17–25              | knobbed    |

https://doi.org/10.1371/journal.pone.0197025.t002
almost entirely brown while still attached to the stem. Conidiomata from leaves, subglobose to oval, immersed, then erumpent, black, up to 150–219 μm wide (n = 10); Conidiogenous cells directly lining the acervular wall, hyaline, cylindrical, annellidic; Conidia: 24–31 × 7.5–10 μm (n = 30), pyriform, curved, four-septate and slightly constricted at the septa, which are darker than the body of the cells; median three cells 15.5–22.5 μm long (n = 30) in total, pigmented; two upper pigmented cells fuscous, darker than lower pigmented cell, 15.5–22.5 μm long (n = 30); apical cell: hyaline, conical with two to four (mostly three) apical appendages arising from the apical crest. Apical appendages typically swollen at the tip, unbranched, filiform, 22–61 μm long (n = 30). Basal cell hyaline, conical, with a single, tubular, unbranched, centric appendage.

Additional specimen examined: DAVFP 11308 (S3 Fig), Cowichan Lake, Vancouver Island, BC, Canada, April 23, 1959, on leaves of *G. shallon* Pursh collected and determined as *Pestalozzia brunneopruinosa* by W. Ziller.

Note: The Holotype was O. S. C. Herb., 8096 in the original description of *Dermatea* by Zeller in 1934 [1]. This description did not mention the color of mature ascospores. DAVFP 11308 collected by Ziller (as *Pestalopezia*) in 1959 contains mature ascomata and is in sufficiently intact state to observe brownish ascospores. However neither sample was suitable for DNA extraction, and hence we established an epitype. Since obtaining cultures that originate from single ascospores is difficult, we initiated our culture (NOF 3176/TAP13K_ca_as2) from a monoconidial isolate that was obtained from hyphae grown from ascospores of a single ascus. We were able to germinate single ascospores ejected from mature ascocarps onto agar, but the resulting germinants failed to grow beyond an initial germ tube. We designated the epitype of *Pestalotiopsis gibbosa* as DAVFP 29689. We consider *P. gaultheriae* Y.M. Zhang, Maharchch. & K.D. Hyde [28] to be a synonym of *P. gibbosa*, but the authors [28] were unable to obtain living cultures from the specimen of *P. gaultheriae*.

**Discussion**

Our morphological observations and sequence results confirm that *Pestalopezia brunneopruinosa* and *Pestalotia gibbosa* are the same fungus. Conidia of *Pestalotia gibbosa* are strikingly similar to those of *Neopestalotiopsis* species because the three median cells of the conidia are versicoloured, and they could be classified into the genus *Neopestalotiopsis* based on morphology. However, in this study, we demonstrate by genomic analysis that *P. gibbosa* should be transferred to *Pestalotiopsis* s. str., even though its sexual morph is an apothecium.

The majority of the more than 200 species associated with the well-known genus *Pestalotiopsis* s. lat. are typified by the asexual morph, while only a few (14) have known sexual states producing perithecial ascocarps typified by the genus *Pestalosphaeria* [7, 21]. Réblová et al. [10] have recommended use of *Pestalotiopsis* rather than *Pestalosphaeria*, but this recommendation did not take into consideration the potential of either *Neopestalotiopsis* or *Pseudopestalotiopsis* also having teleomorphs genetically related to *Pestalosphaeria*; and the small (three known species), obscure genus *Pestalopezia* was not mentioned at all in this recommendation. All species of *Pestalosphaeria* were considered to be linked to *Pestalotiopsis* s. str. after the three genera *Neopestalotiopsis*, *Pseudopestalotiopsis*, and *Pestalotiopsis* were separated from *Pestalotiopsis* s. lat. [7]. Silvério et al [29] in 2016 and Nozawa et al. [17] in 2017, found the sexual morphs of *Neopestalotiopsis* and *Pseudopestalotiopsis*, both in agreement with the description of *Pestalosphaeria*. Hence, they reported that *Pestalotiopsis* s. str., *Neopestalotiopsis*, and *Pseudopestalotiopsis* produce the same sexual morph. However, the relationship of these fungi to *Pestalopezia*, characterized by the production of apothecia, was not considered in these works. In this study, we obtained strains from conidia of *Pestalotia gibbosa* and from ascospores of...
Pestalopezia. In phylogenetic analyses based on ITS, β-tubulin, and tef1, both strains were placed with Pestalotiopsis s. str. (Fig 2) although the morphological characteristics of conidia were strikingly similar to those of conidia of Neopestalotiopsis (Fig 3). Hence, the name of Pestalotia gibbosa should be changed to Pestalotiopsis gibbosa. Although Pestalopezia Seaver 1942 precedes Pestalotiopsis Steyaert 1949, we recommend using Pestalotiopsis s. str. as this name is more widely known and therefore likely to be better accepted. The species name gibbosa (1887) is older than brunneopruinosa (1942). With our strains, P. gaultheriae belongs to same clade with high bootstrap values (MP/ML/NJ: 100/100/100, Fig 2). Pestalotiopsis gaultheriae was established as a new species based on morphology and molecular data of ITS, β-tubulin and tef1 sequences, which were directly obtained from the fungi on a leaf of salal. However, our sequence data demonstrated that P. gaultheriae was a synonym of Pestalotiopsis gibbosa. In sordariomycetes, there is no fungi producing cup-shaped ascomata. According to results of Zhuang et al [30] based on a phylogenetic tree of RNA secondary structures and on the estimated morphologies from their phylogenetic tree, ascomata having exposed hymenia are estimated as ancestral morphs. Even Pestalotiopsis s. lat. produces closed ascomata, and only the clade of Pestalotiopsis gibbosa produces open ascomata, nested among other taxa with closed ascomata. In this study, we were unable to determine whether this is the ancestral morph or a reversion morph. Our results provide the first evidence that Sordariomycetes include species that produce cup-shaped ascomata.

Supporting information

S1 Fig. Specimen of DAVFP 11308. This specimen is preserved in the Forest Pathology Herbarium at the Pacific Forestry Center, Victoria, BC, Canada. (TIF)

S2 Fig. Multiple alignment of ITS sequences among Pestalopezia brunneopruinosa (NOF 3176/TAP13K_ca_as2), Pestalotiopsis gibbosa (NOF 3175/TAP13K_P3), and extract DNA directly from an apothecium on DAVFP 29689. (TIF)

S3 Fig. Morphological characteristics of Pestalotia gibbosa (DAVFP 11308). (A) Apothecia; (B) Acervuli; (C) Asci containing mature ascospores (arrow) on the layer of an apothecium; (D) Asci and ascospores (stained with iodine); (E) Conidial formation on the upper layer of an acervulus; and (F) Conidia. Bars (A), (B): 2 mm, (C): 100 μm, (D)–(F): 20 μm. (TIF)

S1 Table. Morphological comparison of sexual morph of Pestalopezia brunneopruinosa and related species. (DOCX)

Acknowledgments

This research was supported by JSPS KAKENHI Grant Number 25440218 to Kyoko Watanabe.

Author Contributions

Conceptualization: Kyoko Watanabe, Brenda Callan.

Data curation: Kyoko Watanabe, Shunsuke Nozawa.

Formal analysis: Kyoko Watanabe.
Visualization: Kyoko Watanabe, Shunsuke Nozawa.

Writing – original draft: Kyoko Watanabe, Shunsuke Nozawa, Brenda Callan.

Writing – review & editing: Kyoko Watanabe, Tom Hsiang, Brenda Callan.

References

1. Zeller SM. Some new or noteworthy fungi on ericaceous hosts in the Pacific Northwest. Mycologia. 1934; 26(4): 291–304.
2. Harkness HW. Fungi of the pacific coast. Bull Calif Acad Sci. 1887; 2: 438–447.
3. Bonar L. Studies on some California Fungi: II. Mycologia. 1942; 34(2): 180–192.
4. Seaver FJ. Photographs and descriptions of cup-fungi: XXXVI. A new species and genus. Mycologia. 1942; 34(3): 298–301.
5. De Notaris G. Micromycetes italici novi el minus cogniti. Taurini Dec Secundas. Mem Reale Accad Sci Torino. 1841; 3: 80.
6. Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW. Pestalotiopsis revisited. Stud Mycol. 2014; 79: 121–183.
7. Reblova M, Miller AN, Rossman AY, Seifert KA, Crous PW, Hawksworth LD, et al. Recommendations for competing sexual-asexually typified generic names in Sordariomycetes (except Diaporthales, Hypocreales, and Magnaporthales). IMA Fungus. 2016; 7(1): 131–153. https://doi.org/10.5598/imafungus.2016.07.01.08 PMID: 27433444
8. Kirk PM, Stalpers JA, Braun U, Crous PW, Hansen K, Hawksworth DL et al. A without-prejudice list of generic names of fungi for protection under the International Code of Nomenclature for algae, fungi, and plants. IMA Fungus. 2013; 4(2): 381–443. https://doi.org/10.5598/imafungus.2013.04.02.17 PMID: 24563844
9. Kirk PM, Cannon PF, Minter DW, Stalpers JA. Dictionary of the Fungi, 10th Edition. Wallingford: CAB International; 2008.
10. Reblova M, Miller AN, Rossman AY, Seifert KA, Crous PW, Hawksworth LD et al. Estimating the phanerozoic history of the ascomycota lineages: combining fossil and molecular data. Mol Phylogenet Evol. 2014; 78(9): 386–398. https://doi.org/10.1016/j.ympev.2014.04.024 PMID: 24792086
11. Taylor JW. One Fungus = One Name: DNA and fungal nomenclature twenty years after PCR. IMA Fungus. 2011; 2(2): 113–120. https://doi.org/10.5598/imafungus.2011.02.02.01 PMID: 22679595
12. Wingfield MJ, Beer ZW, Slippers B, Wingfield BD, Groenewald JZ, Lombard L, et al. One fungus, one name promotes progressive plant pathology. Mol Plant Pathol. 2012; 13(6): 604–613. https://doi.org/10.1111/j.1364-3703.2011.00768.x PMID: 22146077
13. Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Appl Environ Microbiol. 1995; 61(4): 1323–1330. PMID: 7747954
14. Nozawa S, Yamaguchi K, Yen LTH, Van Hop D, Phay Nyunt, Ando K, Watanabe K. Identification of two new species and a sexual morph from the genus Pseudopezicula. Mycoscience. 2017; 58(5): 328–337. https://doi.org/10.1016/j.myc.2017.02.008
15. Rehener SA, Buckley E. A Beauveria phylogeny inferred from nuclear ITS and EF-1-a sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia. 2005; 97(1): 84–98. https://doi.org/10.3852/mycologia.97.1.84 PMID: 16389960
16. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315–322.
17. Tamaru F, Hinkley CS, Ramprashad N. A comparison of DNA Extraction methods using Petunia hybrida tissues. J Biomol Tech. 2013; 24(3): 113–118. https://doi.org/10.7171/jbt.13-2403-001 PMID: 23997658
21. Maharachchikumbura SSN, Guo LD, Cai L, Chukeatirote E, Wu WP, Sun X, et al. A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. Fungal Divers. 2012; 56(1): 95–129. https://doi.org/10.1007/s13225-012-0198-1

22. Katoh K, Rozewicki J, Yamada KD. 2017 MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform. 1–7. https://doi.org/10.1093/bib/bbw003 PMID: 26868358

23. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 1985; 39 (4): 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x PMID: 28561359

24. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol. 2016; 33(7):1870–4. https://doi.org/10.1093/molbev/msw054 PMID: 27004904

25. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4(4): 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454 PMID: 3447015

26. Kimura M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980; 16(2): 111–120. https://doi.org/10.1007/bf01731581 PMID: 7463489

27. Page RDM. TreeView: An application to display phylogenetic trees on personal computers. Comput Appl Biosci. 1996; 12(4): 357–358.

28. Zhang YM, Maharachchikumbura SSN, Tian Q, Hyde KD. *Pestalotiopsis* species on ornamental plants in Yunnan Province, China. Sydowia 2013; 65(1): 113–128.

29. Silvério ML, Calvacanti MAQ, Silva GA, Oliveira RJV, Bezerra JL. A new epifoliar species of *Neopestalotiopsis* from Brazil. Agrotropica. 2016; 28(2): 151–158. https://doi.org/10.21757/0103-3816.2016v28n2p151-158

30. Zhuang W-Y, Liu C-Y. What an rRNA secondary structure tells about phylogeny of fungi in Ascomycota with emphasis on evolution of major types of ascus. PLoS ONE. 2012; 7: e47546. https://doi.org/10.1371/journal.pone.0047546 PMID: 23110078