Mycologia, 99(1), 2007, pp. 144–151.

Three new species of *Luttrellia* from temperate and tropical freshwater habitats

Astrid Ferrer[1]
Carol A. Shearer

Department of Plant Biology, University of Illinois, 265 Morrill Hall, 505 South Goodwin Avenue, Urbana, Illinois 61801

Abstract: Three pyrenomycetes collected from woody debris submerged in freshwater habitats are described as new species of *Luttrellia: L. guttulata* sp. nov., *L. halonata* sp. nov. and *L. parvulospora* sp. nov. A collection of *L. estuarina*, the type species of the genus, having ascospores surrounded by a gelatinous sheath, a characteristic not observed in the type specimen, is reported. The genus description is emended to include fungi with 4- or 8-spored asci and ascospores with or without a gelatinous sheath. *Luttrellia* is distinguished from other genera in the *Halosphaeriaceae* by hyaline, phragmoseptate, and thick-walled ascospores with or without a gelatinous sheath.

Key words: aquatic, euascomycetes, fungal systematics

INTRODUCTION

The genus *Luttrellia* (Halosphaeriaceae, Halosphaeriales) was established by Shearer (1978) to accommodate a single species, *L. estuarina*. *Luttrellia* was characterized by superficial to partially immersed, globose to subglobose, membranous ascomata, clavate to cylindrical, early deliquescent, 4-spored asci, and hyaline, multiseptate ascospores. During our study of freshwater euascomycetes through North, Central and South America, we encountered three ascomycetes with morphological characteristics similar to those of *L. estuarina* but differing in ascus and ascospore morphology and size, and they therefore are described as new species.

MATERIAL AND METHODS

Samples of submerged partially decomposed woody debris were collected from rivers and streams and placed into Ziploc™ plastic bags containing paper towels to absorb excess water. Samples were incubated in the laboratory in plastic boxes containing moistened paper towels at ambient temperature (about 24 C) and were examined with a dissecting microscope immediately after collection and periodically for 9 mo.

Ascomata were placed on a cover glass on a microscope slide in a drop of distilled water and opened to release asci and ascospores with dissecting needles. Aqueous nigrosin or India ink was added to the water mounts to reveal appendages or gelatinous sheaths on the ascospores. Distilled water was replaced with glycerin for preservation with the double cover glass method (Volkmann-Kohlmeyer and Kohlmeyer 1996). Measurements of the hamathecium, asci and ascospores were made from ascomata in distilled water or fixed in glycerin.

Additional ascomata were fixed and sectioned with a modification of Huhndorf's technique (Huhndorf 1991, Fallah and Shearer 2001). Digital images were obtained with a Spot RT digital camera using an Olympus microscope equipped with Nomarski interference optics. Specimens are deposited in the Herbarium of the University of Illinois at Urbana Champaign (ILL), in the Herbarium of the University of Panama (PMA), and in the Herbarium of the Pontificia Universidad Católi of Ecuador (PUCE).

In the specimen examined section, information about country, latitude and longitude, as well as water temperature and pH are presented. Collector's names are abbreviated: JC (J. Campbell), JLC (J. L. Crane), AF (A. Ferrer), WLH (W.L. Hurley), CMP (C. M. Pringle), KR (K. Robertson), CAS (C. A. Shearer), MJW (M.J. Wetzel).

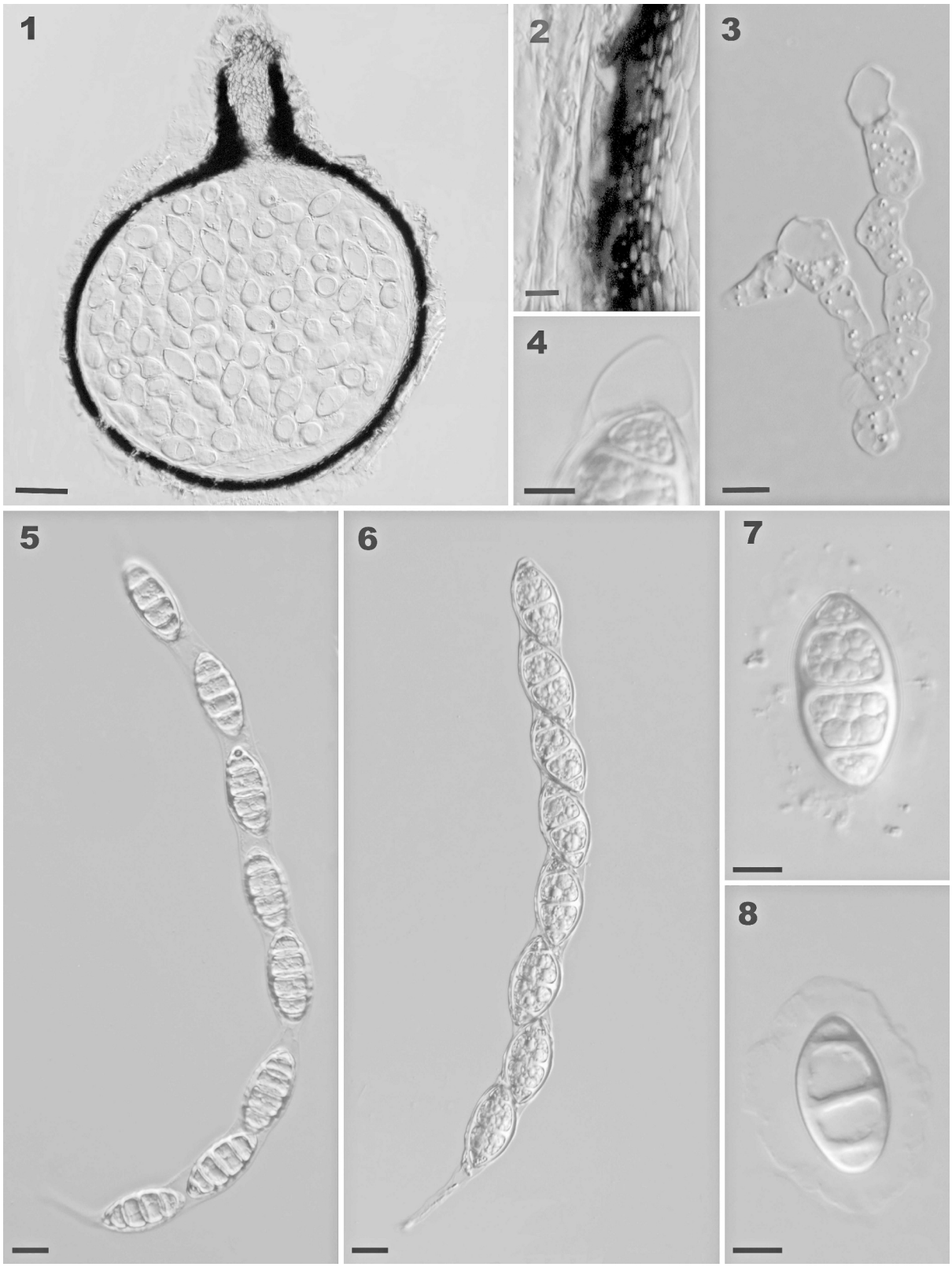
TAXONOMY

Luttrellia guttulata A. Ferrer et Shearer, sp. nov.
FIGS. 1–8

Ascomata 380–425 × 490–670 μm, partim immersa vel immersa, globosa vel subglobosa, membranacea, nigra, ostiolati. Collum 100–170 × 70–80 μm, cylindricum, atrobrunneae. Peridium 12–20 μm latum, 8–10 cellulae crassum. Catenophysibus 10–14 μm latae, hyalinae. Asci 320–350 × 24 μm, unitunicati, cylindrici, tenuitunicati, sine poro apicali, octospori, uniseriati, deliquescentes. Ascosporae 36–48 × 20–25 μm, ellipsoideae-fusiformes, hyalinae, 3–5-septatae, ad septum non constrictae, pachydermaticae, tunica gelatinosa praeditae.

Ascomata on wood 380–425 × 490–670 μm, partially immersed to immersed in the substrate, globose to subglobose, membranous, black, ostiolate. Neck 100–170 × 70–80 μm, cylindrical, dark brown (FIG. 1). Peridium 12–20 μm thick, composed of 8–10 cell layers, outer region of dark, elongated cells, inner region of hyaline, elongated to isodiametric cells (FIG. 2). Hamathecium of catenophyses, 10–14 μm wide, septate, hyaline (FIG. 3). Asci 320–350 × 24 μm, unitunicate, cylindrical, thin-walled, lacking

Accepted for publication 29 September 2006.
[1] Corresponding author. E-mail: aferrer@life.uiuc.edu



FIGS. 1–8. *Luttrellia guttulata* (AF181-1). 1. Longitudinal median section through an ascoma. 2. Longitudinal median section though peridium. 3. Catenophyses. 4. Ascus tip showing the absence of pore or other apical structures. 5–6. Asci. 7. Ascospore with sheath mounted in distilled water. 8. Sheathed ascospores fixed in glycerin. Bars: 1 = 50 μm; 5–6 = 20 μm; 2–4, 7, 8 = 10 μm.

an apical pore and apical structures (FIG. 4), 8-spored, uniseriate to overlapping uniseriate, early deliquescent (FIGS. 5, 6), asci elongating in water. Ascospores 36–48 × 20–25 μm (mean = 39.95 × 20.69 μm, n = 30), broadly ellipsoidal-fusiform to ellipsoidal, hyaline, 3-septate, occasionally 5-septate, not constricted at septa, thick-walled (2.0–2.5 μm), surrounded by a gelatinous sheath about 6–10 μm wide (FIGS. 7–8). Ascospores accumulate at tip of the neck after discharge.

Specimens examined. PANAMA: COLON, Barro Colorado National Monument, Wheeler Stream, 9°10′N, 79°51′W, water temperature 26 C, pH 6, on submerged decorticated wood, 18 Aug 2004, *AF, AF181-1.* (ILL). HOLOTYPE.

Additional specimens examined. COSTA RICA: HEREDIA, La Selva Biological Station, Piper, on submerged decorticated wood, 6 Feb 2001, *CMP, A544-1.*

Known distribution. Costa Rica, Panama

Etymology. Guttulatus = ''apparently sprinkled with dots of oil or resin'', in reference to oil deposits that look like bubbles in the ascospores.

Comments. The general characteristics of *L. guttulata* fit within the concept of the genus *Luttrellia.* These include dark, membranous ascomata, thin-walled deliquescent asci and thick-walled, hyaline, multiseptate ascospores (Shearer 1978). Nonetheless asci contain eight ascospores, instead of four as in the type species of the genus, *L. estuarina,* and ascospores develop septa before asci deliquesce.

Distinctive features of *L. guttulata* are the presence of a prominent gelatinous sheath around the ascospores and mostly 3-septate ascospores. These ascospore characteristics are similar to those of *L. halonata*, a new species described herein but which has 4-spored asci and 5-septate ascospores.

Luttrellia halonata A. Ferrer et Shearer, sp. nov. FIGS. 9–15

Ascomata 250–300 × 230–280 μm, partim immersa vel immersa, globosa vel subglobosa, membranacea, nigra, ostiolati. Collum 50 × 55 μm, cylindricum, atrobrunneae. Peridium 10–15 μm latum, 5–6 cellulae crassum. Catenophysibus 15–18 μm latae, hyalinae. Asci 145–165 × 25–32 μm, unitunicati, cylindrici, tenuitunicati, sine poro apicali, quadrisporis, uniseriati, deliquescentes. Ascosporae 36–52 × 26–32 μm, ellipsoideae-fusiformes, hyalinae, 5-septatae, ad septum non constrictae, pachydermaticae, tunica gelatinosa praeditae.

Ascomata on wood 250–300 × 230–280 μm, partially immersed to immersed in the substrate, globose to subglobose, membranous, black, ostiolate. Neck 50 × 55 μm, cylindrical, dark brown (FIG. 9). Peridium 10–15 μm thick, composed of 5–6 cell layers of dark, globose to elongated cells (FIG. 10). Hamathecium of catenophyses, 15–18 μm wide, septate, hyaline (FIG. 11). Asci 145–165 × 25–32 μm, unitunicate, cylindrical, short pedicellate, thin-walled, lacking an apical pore, 4-spored, uniseriate, irregularly arranged, early deliquescent (FIG. 12). Ascospores 36–52 × 26–32 μm (mean = 44.5 × 28.0 μm, n = 30), broadly ellipsoidal-fusiform to ellipsoidal, hyaline, 5-septate, not constricted at septa, thick-walled (3.0–5.0 um), surrounded by a thick, gelatinous sheath about 5–10 μm wide (FIGS. 13–15). Ascospores accumulate at tip of the neck after discharge.

Specimens examined. ECUADOR: NAPO, Yasuni National Park, Tiputini River, 9°10′N, 79°51′W, water temperature 26 C, pH 5.5–6, on submerged decorticated wood, 4 Apr 2004, *AF, AF134-1.* (ILL). HOLOTYPE.

Known distribution. Ecuador

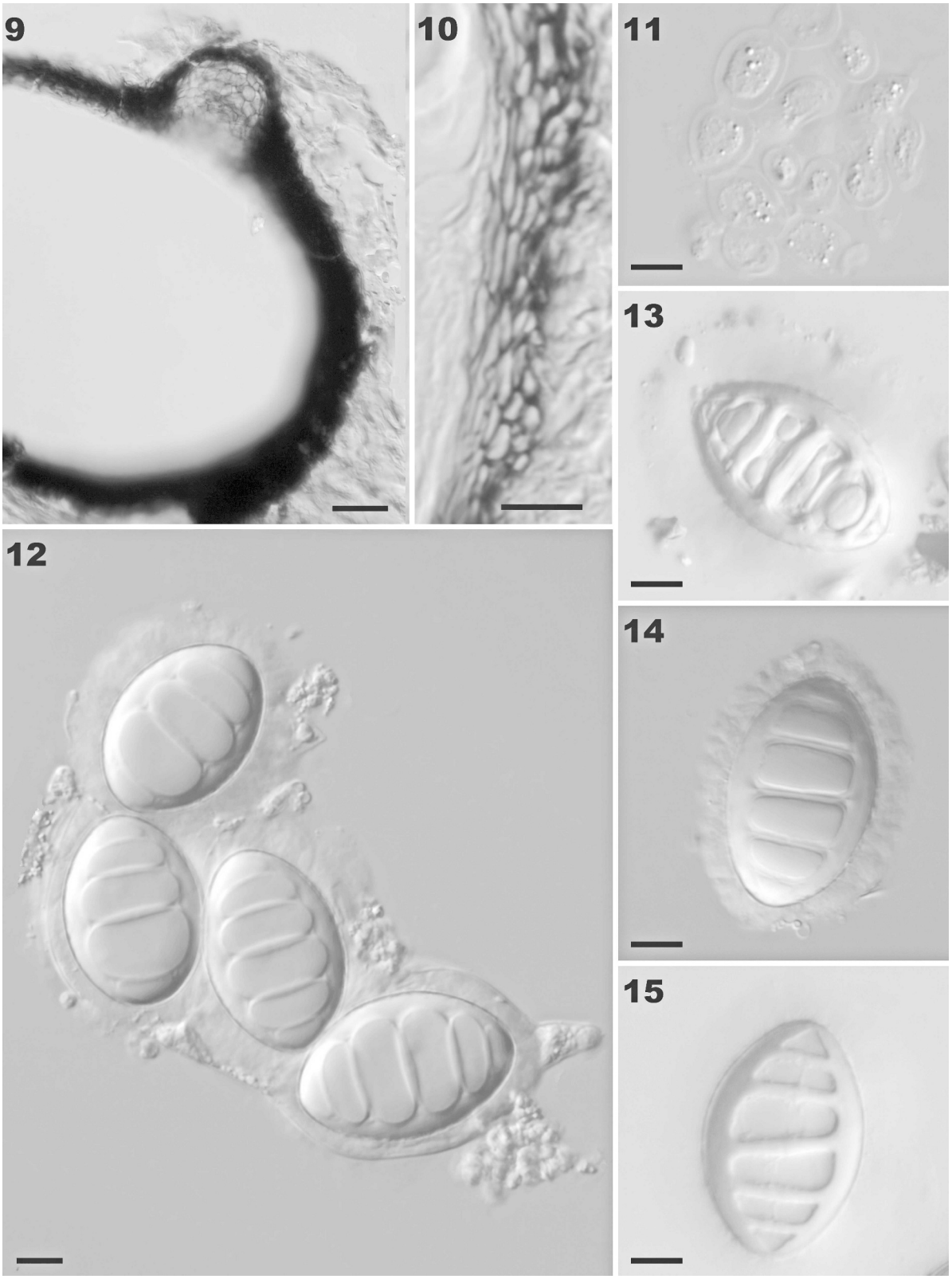
Etymology. Halonatus = ''surrounded by an outer circle'', in reference to the sheath that surrounds the spore like a halo.

Comments. Luttrellia halonata is morphologically similar to the type species of *Luttrellia*, *L. estuarina*, with dark ascomata, thin-walled asci lacking an apical pore, 4-spored asci and thick-walled, hyaline, multiseptate ascospores. This new species differs from *L. estuarina* however in having wider and shorter ascospores, and 5- rather than 5–9-septate ascospores. In addition ascospores of *L. halonata* are surrounded by a distinctive thick, muscilaginous sheath, while those of *L. estuarina* lack a sheath or have a thin one (FIGS. 23–26).

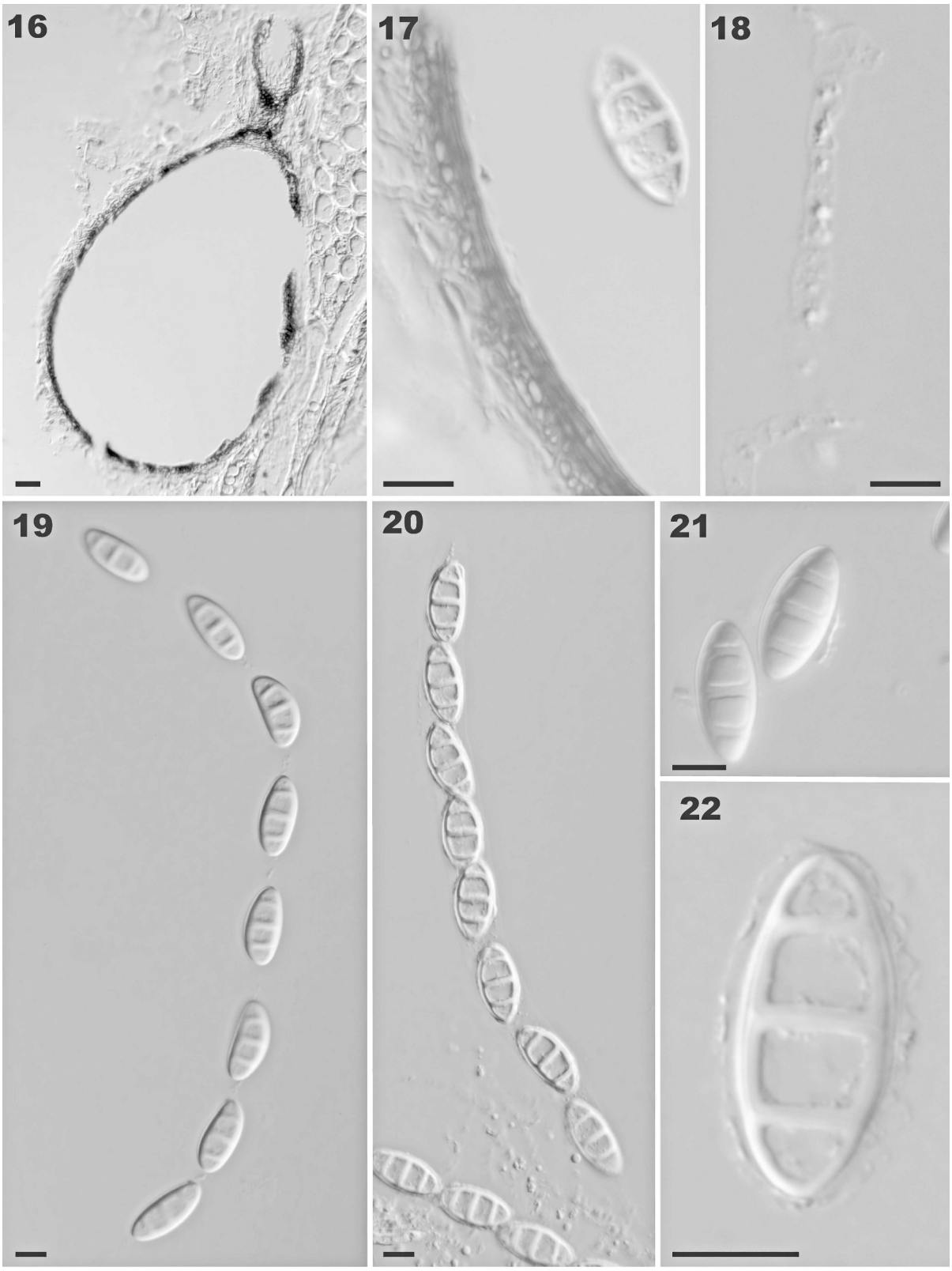
Luttrellia parvulospora Shearer et A. Ferrer sp. nov. FIGS. 16–22

Ascomata 220–375 × 180–195 μm, partim immersa vel immersa, subglobosa vel obpyriformia, membranacea, nigra, ostiolati. Collum 100–130 × 40–80 μm, cylindricum, atrobrunneae. Peridium 7–10 μm latum, 3–5 cellulae crassum. Catenophysibus 5–10 μm latae, hyalinae. Asci 210–230 × 12–14 μm, unitunicati, cylindrici, tenuitunicati, sine poro apicali, octospori, uniseriati, deliquescentes. Ascosporae 23–27 × 10–12 μm, ellipsoideae, hyalinae, 3-septatae, ad septum non constrictae, pachydermaticae, tunica gelatinosa praeditae.

Ascomata on wood 220–375 × 180–195 μm, partially immersed to immersed in the substrate, subglobose to obpyriform, membranous, black, ostiolate. Neck cylindrical, dark brown, 100–130 × 40–80 μm (FIG. 16). Peridium 7–10 μm thick, composed of 3–5 cell layers of dark, elongated to isodiametric cells (FIG. 17). Hamathecium of catenophyses, 5–10 μm wide, septate, hyaline (FIG. 18). Asci 210–230 × 12–14 μm, unitunicate, cylindrical, thin walled, lacking an apical pore and other apical structures, 8-spored, uniseriate, early deliquescent (FIGS. 19 and 20).



FIGS. 9–15. *Luttrellia halonata* (AF134-1). 9. Longitudinal median section through ascoma. 10. Longitudinal median section though peridium. 11. Catenophyses. 12. Ascus with four ascospores. 13–14. Ascospores mounted in distilled water illustrating gelatinous sheath. 15. Ascospores fixed in glycerin. Bars: 9 = 20 μm; 10–15 = 10 μm.



FIGS. 16–22. *Luttrellia parvulospora* (A-276-5). 16. Longitudinal median section through ascoma. 17. Longitudinal median section though peridium. 18. Catenophysis. 19–20. Asci. 21–22. Ascospores. Bars: 16 = 20 μm; 17–22 = 10 μm.

Ascospores 23–27 × 10–12 μm (mean = 25.3 × 11 μm, n = 30), ellipsoidal, hyaline, 3-septate, not constricted at septa, thick-walled (up to 2.0 um), surrounded by a gelatinous sheath about 3–7 μm wide (FIGS. 21–22). Ascospores accumulate at the tip of the neck after discharge.

Specimens examined. USA: MISSISSIPPI, Wolf River, 30°21′25″N, 89°17′12″W, water temperature 24 C, pH 6.5, on submerged decorticated wood, 16 Jun 1997, *KR, A-276-5.* (ILL). HOLOTYPE.

Additional specimens examined. USA: LOUSIANA, Creek in Washington Parish, 30°43′38″N, 90°05′03″W, water temperature 26 C, 6.5 pH, on submerged decorticated wood, 27 Aug 1997, *KR, A-276-3.* Creek in Washington Parish, 30°43′38″N, 90°05′03″W, water temperature 22 C, 6.5 pH, on submerged decorticated wood, 27 Aug 1997, *KR, A-276-4.* FRENCH GUIANA: Degrad Eskol, Crique Gabrielle, Commune Noura, 04°43′N, 52°17′W, on submerged decorticated wood, 29 Sep 1995, *JLC, A-276-2.*

Known distribution. French Guiana, USA (LA, MS)

Etymology. Parvus = ''little, small''; *spora* = ''spore'', in reference to the small size of the spore.

Comments. L. parvulospora is similar to *L. guttulata*, in having the same number of septa, and asci with eight ascospores. The ascospores are the smallest in the genus and differ in shape from those *of L. guttulata.*

Luttrellia estuarina Shearer, Mycologia 70:692–697. 1978. FIGS. 23–26

Ascomata 207–416 × 277–455 μm, globose to subglobose, membranous, black, ostiolate. Neck 248–822 × 25–40 μm, cylindrical, hyaline to dark brown. Asci 103–158 × 12–26 μm, unitunicate, clavate to cylindrical when immature, elongated at maturity, thin-walled, lacking an apical pore and other apical structures, 4-spored, uniseriate, early deliquescent. Ascospores 42–55 × 16–22 μm, elliptical, hyaline, 5–9-septate, not constricted at septa, thick-walled, surrounded by a thin gelatinous sheath up to 3 μm wide (FIGS. 24–26). Ascospores accumulate in a mass at tip of the neck after discharge.

Specimens examined. CANADA: MANITOBA, Whiteshell Provincial Park, Rennie River at junction with Route 307. River drains Heart Lake, 50°06′58″N, 95°37′53″W, water temperature 24 C, pH 5, on submerged decorticated wood, 23 Jul 1992, *JLC, CAS,* & *WLH, A-162-4.* USA: COLORADO, Larimer County, small river, water temperature 17 C, pH 6, on submerged decorticated wood, 5 May 1997, KR, A-162-7. Decker at swampy fork in River Platt, 39°15′N, 105°14′W, water temperature 19 C, pH 5.5–6, on submerged decorticated wood, 26 Jul 1998, *CAS, A-162-5.* FLORIDA, Monroe County, Sweetwater Stand at junction with Route 94, about 5.9 miles from Monroe, 25°46′57″N, 81°5′37″W, water temperature 24 C, pH 6.4, on submerged decorticated wood, 13 May 1993, *JLC* & *CAS, A-162-1.* ILLINOIS, Union County, Wolf Lake, 37°34′36″N, 89°26′20″W, water temperature 12 C, pH 6, on submerged decorticated wood, 9 Mar 1994, *JLC* & *CAS, A-162-3.* MARYLAND, Calvert County, on balsa wood retrieved from the Patuxent River at Lower Marlboro, 16 Dec 1969, *CAS, CS-80-2.* Calvert County, on balsa wood retrieved from the Patuxent River at Lower Marlboro, 6 May 1969, *CAS, CS-80-3.* Calvert County, on balsa wood retrieved from the intake canal of the Potomac Electric Power Company (PEPCO) generating plant, at Chalk Point, on the Patuxent River, 26 Jul 1973, *CAS, CS-80-4* (HOLOTYPE). MINNESOTA: Itasca County, Bellow Lake, about 31 miles north on Route 38 from Route 2, 47°40′4″N, 93°42′27″W, water temperature 8 C, pH 6, on submerged decorticated wood, 23 Oct 1993, *JLC & CAS.* NORTH CAROLINA, Great Smoky Mountains National Park, on submerged decorticated wood, 21 Apr 2002, *MJW, A-162-8.*

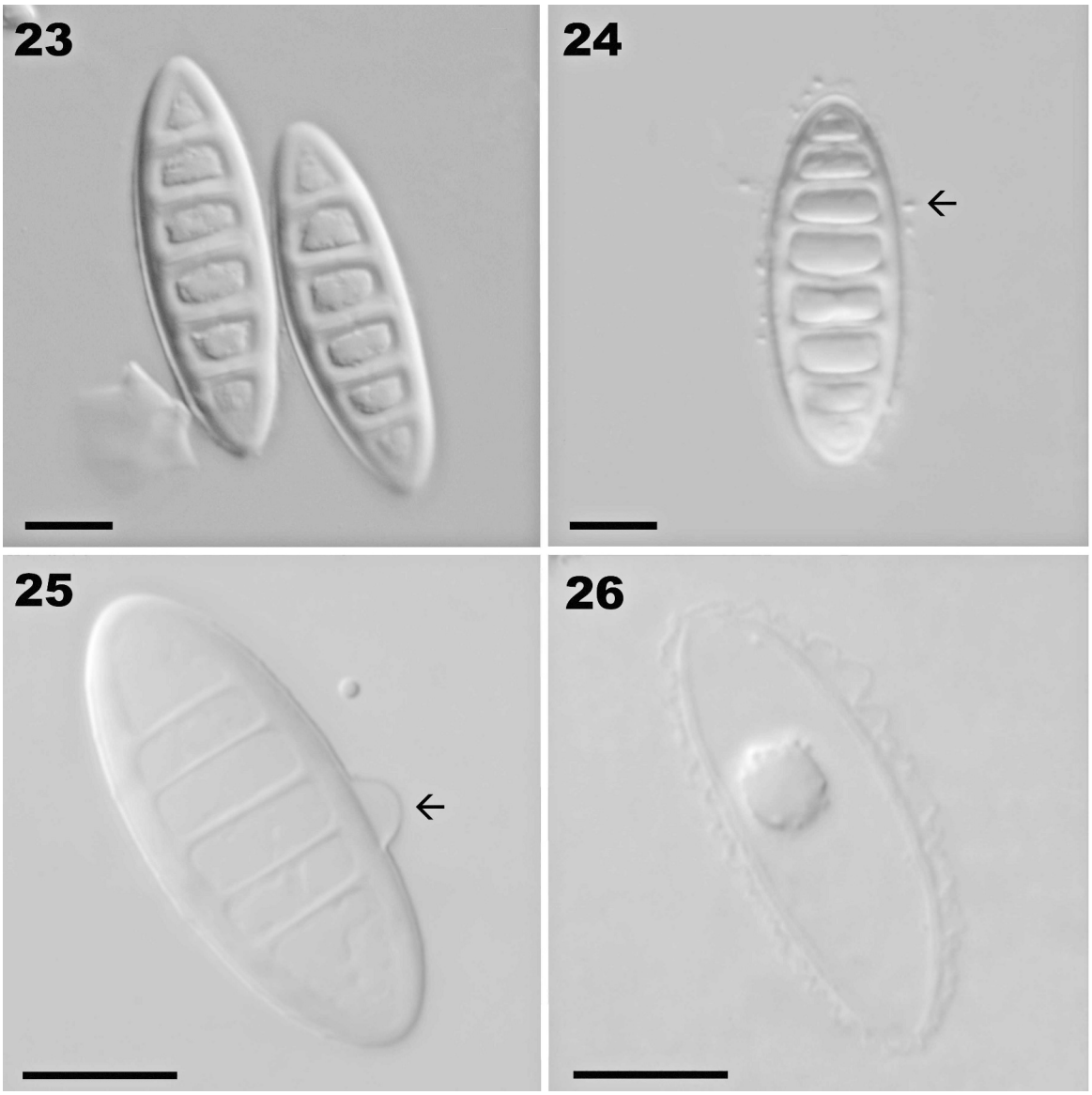
Known distribution. CANADA, HONG KONG, USA (CO, FL, IL, MD, MN, NC)

Comments. L. estuarina originally was described as lacking appendages or a sheath (FIG. 23), nonetheless subsequent collections showed the presence of a thin sheath around the ascospores (FIGS. 24–26).

L. estuarina is the most widely distributed species of *Luttrellia*, with collections from Manitoba, Canada, to Florida, USA. It also has been reported from Hong Kong (Tsui et al 2000). Although originally described from brackish water *L. estuarina* occurs on submerged wood in freshwater lotic and lentic habitats (http://www.life.uiuc.edu/plantbio/fungi/).

DISCUSSION

The genus *Luttrellia* initially was separated from other genera in the Halosphaeriaceae by its ascospore morphology, thick-walled, phragmoseptate and lacking appendages or a sheath. In our study of *Luttrellia* we have observed that all the species have ascospores that are surrounded by a gelatinous sheath. This type of ascospore modification is uncommon in the *Halosphaeriaceae* (Jones 1995) because most taxa have polar or equatorial appendages. However care must be used in the interpretation of this taxonomic character because in both *L. estuarina* and *L. guttulata* ascospores with and without sheathes were observed from the same collections. The presence of other ascospore modifications also may be variable within the Halosphaeriaceae. For example collections of *Nais inornata* and *Aniptodera chesapeakensis* (Shearer and Crane 1978, 1980) have been described with appendaged and unappendaged ascospores. It has been suggested that this variation might reflect genetic differences among populations, but it also might be due to environmental influence on gene expression. On the other hand it simply might reflect



FIGS. 23–26. *Luttrellia estuarina*. 23. Ascospores from the holotype (CS-80-4). 24–25. Ascospores (A-165-2). 26. Released sheath from ascospore (A-165-2). Bars = 10 μm.

the stage of development at which the fungi are observed (Pang et al 2003). Hyde et al (1999) concluded that the presence or absence of appendages is not a reliable character for the delineation of genera, given the ephemeral nature of appendages in some genera.

Luttrellia species are similar with respect to various traits, such as globose to subglobose, membranous, brown to brown black ascomata and thick walled, hyaline phragmoseptate ascospores. The hamathecium consists of catenophyses. Nonetheless *Luttrellia* species can be identified based on the number of ascospores per asci and by ascospore morphology and septation (TABLE I). All *Luttrellia* species described thus far were collected from fresh and brackish water habitats. The most widely distributed species is *L. estuarina*, which has been collected from lentic to lotic habitats, and from temperate and tropical habitats. The other species are uncommon and may have more restricted distributions. Most of the ascomata of *Luttrellia* are immersed in the substratum; the accumulation of ascospores at the tip of the neck that look like a bright mass on the substratum may help locate otherwise inconspicuous species.

Luttrellia Shearer emend.

Ascomata superficial to immersed, globose, subglobose to obpyriform, membranous, hyaline becoming black at maturity, ostiolate. Neck short to long,

TABLE I. Synopsis of *Luttrellia* species

	L. estuarina	*L. guttulata*	*L. halonata*	*L. parvulospora*
Ascomata	227–416 × 277–455 μm	380–425 × 490–670 μm	250–300 × 230–280 μm	220–375 × 180–195 μm
Catenophyses	+	+	+	+
Asci	103–158 × 12–26 μm	320–350 × 24 μm	145–165 × 25–32 μm	210–230 × 12–14 μm
Spores/Asci	4-spored	8-spored	4-spored	8-spored
Ascospore septation	5–9-septate	Mostly 3-septate (3–5)	5-septate	3-septate
Ascospore size	42–55 × 16–22 μm	36–48 × 20–25 μm	36–52 × 26–32 μm	23–27 × 10–12 μm
Sheath	Up to 3 μm	6–10 μm	5–10 μm	3–7 μm

cylindrical, hyaline to dark brown, periphysate. Asci originating from a restricted area at the base of perithecium in a single fascicle, surrounded by catenophyses, clavate asci to cylindrical when immature, elongated and cylindrical at maturity, thin-walled, unitunicate, 4- or 8-spored, early deliquescent. Ascospores hyaline, septate, thick walled, with or without a gelatinous sheath, lacking appendages.

ACKNOWLEDGMENTS

We thank Huzefa Raja and Jim Dalling for reviewing the manuscript before submission. We also thank our collectors, JC, JLC, WLH, CMP, KR and MJW, for their help. Appreciation is expressed to the STRI and PUCE for their logistic support to collect in Panama and Ecuador. Financial support by the National Science Foundation (NSF Grant No. DEB 0316496) is gratefully acknowledged.

LITERATURE CITED

Fallah PM, Shearer CA. 2001. Freshwater ascomycetes: new or noteworthy species from north temperate lakes in Wisconsin. Mycologia 93(3):566–602.

Jones EBG. 1995. Ultrastructure and taxonomy of the aquatic ascomycetous order *Halosphaeriales*. Can J Bot 73:S790–S801.

Huhndorf SM. 1991. A method of sectioning ascomycete herbarium specimens for light microscopy. Mycologia 83:520–524.

Hyde KD, Ho W-H, Tsui KM. 1999. The genera *Aniptodera*, *Halosarpheia*, *Nais* and *Phaeonectriella* from freshwater habitats. Mycoscience 40:165–183.

Pang K-L, Vrijmoed LLP, Kong RYC, Jones EBG. 2003. *Lignincola* and *Nais*, polyphyletic genera of the Halosphaeriales (Ascomycota). Mycol Prog 2(1):29–36.

Shearer CA. 1978. Fungi of the Chesapeake Bay and its tributaries VII. *Luttrellia estuarina* gen. et sp. nov. (Ascomycetes) Mycologia 70(3):692–697.

———, Crane JL. 1978. The distribution of *Nais inornata*, a facultative marine Ascomycete. Mycotaxon 7:443–458.

———, ———. 1980. Fungi of the Chesapeake Bay and its tributaries VIII. Aquatic ascomycetes with unfurling appendages. Bot Mar 23:607–615.

Tsui CKM, Hyde KD, Hodgkiss IJ. 2000. Biodiversity of fungi on submerged wood in Hong Kong streams. Aqua Microb Ecol 21(3):289–298.

Volkmann-Kohlmeyer B, Kohlmeyer J. 1996. How to prepare truly permanent microscope slides. Mycologist 10: 107–108.