Cytology of *Poronia oedipus* and *P. punctata*[1]

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The chromosome numbers of *Poronia oedipus* and *Poronia punctata* are $n = 7$, based on counts made of pachytene and metaphase chromosomes. Mature ascospores of *P. oedipus* are binucleate; those of *P. punctata* are quadrinucleate. The taxonomic implications of the cytological results are discussed.

Introduction

Poronia Willd. ex S. F. Gray is a xylariaceous genus defined primarily by nail-shaped stromata and coprophilous habit (4). A number of xylariaceous fungi have characteristics reminiscent of both *Poronia* and of *Xylaria* Hill ex Grev., i.e., flat-topped stromata with lignicolous habit or subglobose stromata with coprophilous habit. Dennis (3) has stressed the coprophilous habit in separating these "borderline" fungi: forms with flat-topped stromata occurring on wood are assigned to *Xylaria* (= *Xylosphaera* Dumortier); forms with subglobose stromata occurring on dung are assigned to *Podosordaria* Ellis and Holway. Morgan-Jones and Lim (8) have recently followed Dennis' logic in assigning a flat-topped lignicolous form to *Xylaria*.

It is of interest to determine if *Poronia* and *Xylaria* can be separated on cytological and other features. Two species of *Xylaria* have been studied cytologically (12, 13). Nuclear phenomena in two undoubted *Poronia* species (*P. oedipus* (Mont.) Mont. and the type, *P. punctata* (L. ex Fr.) Fr.) are reported herein.

Materials and Methods

Stromata of *Poronia oedipus* and *P. punctata* were grown in pure agar culture and harvested periodically for cytological study. The origin of the *P. oedipus* culture and techniques for culturing it have been discussed (7). A culture of *P. punctata* was kindly sent me by Prof. George Carroll, University of Oregon. Professor Carroll isolated the fungus from *Bos* dung collected in Mexico. The fungus fruits well on several media, as will be discussed elsewhere. Fixation, storage, cytological staining by an HCl–Giemsa procedure, and microscopic and photographic apparatus have been described (11).

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Results

Poronia oedipus

Asci of *Poronia oedipus* develop from croziers. Croziers are small and are obliterated by developing asci and by proliferation; they are difficult to demonstrate. Highly contracted bivalents were seen in the smallest asci; it is probable that synapsis occurs in the crozier penultimate cell. As the ascus increases in length and breadth, the bivalents also elongate. Eventually, they attain the classical pachytene morphology (Figs. 1–4). In a very few cases all of the bivalents could be followed for their entire length (Fig. 1). In most preparations one to several chromosomes could be identified. The haploid chromosome number apparently is 7. The individual chromosomes are labelled in Fig. 1 and drawn from tracings in Figs. 1*o*–7*o*.

It was determined early in the investigation that $n = 7$ or 8. Many additional preparations were examined over an 8-month period to determine whether the nucleolar organizer (chromosome 1 in Fig. 1 and Fig. 1*o*) is one chromosome or is a constant terminal association of two chromosomes. I am now satisfied that the nucleolar organizer is one chromosome, the bend possibly representing the centromere region. Moreover, the length of that chromosome (2 to 2.5 times the length of the next longest bivalent) suggests that it might have originated from a fusion of two telocentrics of about equal length.

It was not possible to do a complete karyotype analysis. Centromere positions are uncertain; relative arm lengths could not be established. Metaphase chromosomes (see later), because of their extreme contraction, were not useful in a morphological analysis.

Metaphase–anaphase configurations were infrequent at both meiotic and mitotic divisions and, when observed, were usually indistinct.

Usually, there appeared to be seven chromosomes and, sometimes, the remnants of the nucleolus (Fig. 5).

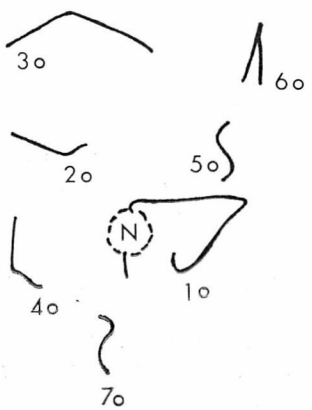
Following the two divisions of meiosis and the mitosis, ascospores are formed around each of the eight resultant nuclei. Immediately after, or perhaps during, ascosporogenesis the single nucleus of each ascospore divides mitotically. This division seems synchronized among the spores of an ascus; all of the spores of a given ascus become binucleate at about the same time (Fig. 6). Maturing ascospores are binucleate (Fig. 7) and remain so until mature dark wall pigmentation makes observation difficult (Fig. 8).

Not infrequently, asci containing fewer than eight spores were observed. In a few cases enucleate ascospores were seen. The contents of one perithecium showed over 50% of asci that had double apical portions; the ascus appeared to branch about midway from the base and looked roughly like a pair of trousers (Fig. 9). None of these anomalous asci showed spores, but showed abnormal numbers of nuclei of various sizes and, apparently, different numbers of chromosomes. Interestingly, the other perithecia from the stroma that bore the aberrant one showed normal asci.

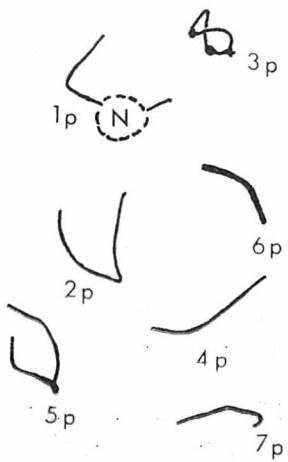
Poronia punctata

Asci of *Poronia punctata* arise from conspicuous croziers (Figs. 10, 11). Synapsis of highly contracted homologues occurs in the crozier penultimate cell. As the ascus enlarges in length and breadth the chromosomes elongate until they reach the classical pachytene morphology (Figs. 12–14). In a few preparations all of the bivalents could be followed for their entire length. There appeared to be seven bivalents. The individuals are labelled in Fig. 13 and drawn from tracings in Figs. 1*p*–7*p*.

The chromosome number of *P. punctata* was somewhat easier to establish than that of *P. oedipus* (see earlier) owing to the large number of metaphase–anaphase figures encountered (see later). Nonetheless, pachytene figures often showed what apparently were eight bivalents. The chromosome labelled 5 in Fig. 13 and Fig.

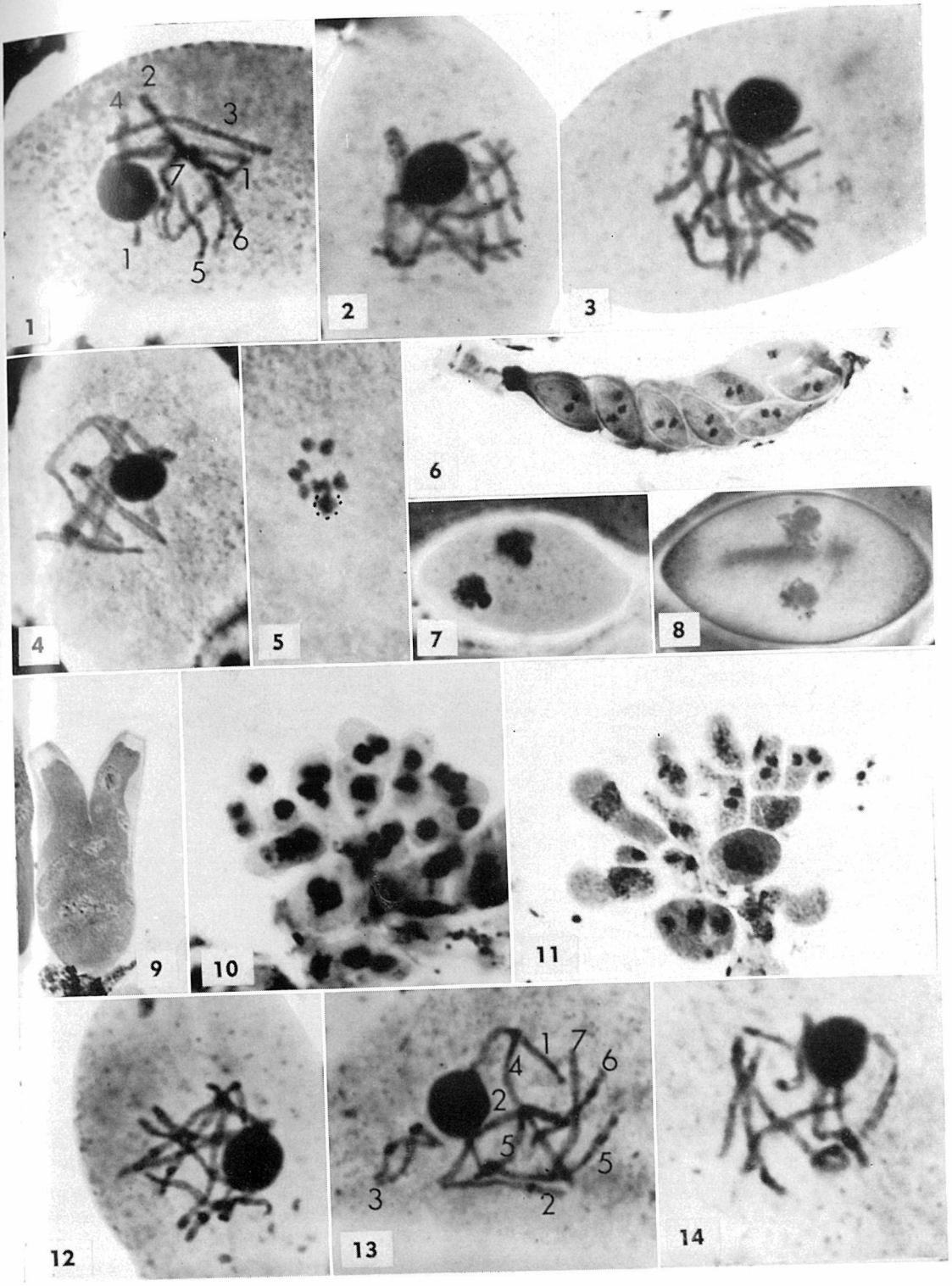


FIGS. 1*o*–7*o*. Pachytene chromosomes of *Poronia oedipus* traced from photographic negatives at about × 2100. An individual chromosome (bivalent) corresponds to that with same number in Fig. 1.



FIGS. 1*p*–7*p*. Pachytene chromosomes of *Poronia punctata* traced from photographic negatives at about × 2100. An individual chromosome (bivalent) corresponds to that with same number in Fig. 13.

FIGS. 1–14. *Poronia oedipus* and *Poronia punctata*. Figs. 1–9. *Poronia oedipus*. 1. Pachytene. All seven bivalents can be seen. Bivalent number 4 (out of focus for a part of its length) lies atop the nucleolus, crossing it from 12 o'clock to 4 o'clock. Individual bivalents are drawn in Figs. 1*o*–7*o* and can be compared with this figure. 2–4. Pachytene. Pairing of homologues is particularly evident in Fig. 4. 5. Metaphase I. Seven bivalents and the remnants of the nucleolus (dotted line) still attached to the organizer. 6. Ascus showing seven (of eight) binucleate ascospores. 7. Young binucleate ascospore. 8. Maturing binucleate ascospore. Note germ slit and dark wall pigmentation. 9. Anomalous ascus with two apical parts. Figs. 10–14. *Poronia punctata*. 10. Croziers and ascogenous hyphae. 11. Croziers, young asci, and portions of the underlying ascogenous system. 12–14. Pachytene. All seven bivalents numbered in Fig. 13. Individual bivalents are drawn in Figs. 1*p*–7*p*. Magnifications: Fig. 9, ca. × 300; Fig. 6, ca. × 700; Fig. 11, ca. × 1100; Fig. 8, ca. × 1700; Figs. 5, 7, 10, ca. × 1900; Figs. 1, 3, 4, 12, 13, ca. × 2100; Figs. 2, 14, ca. × 2300.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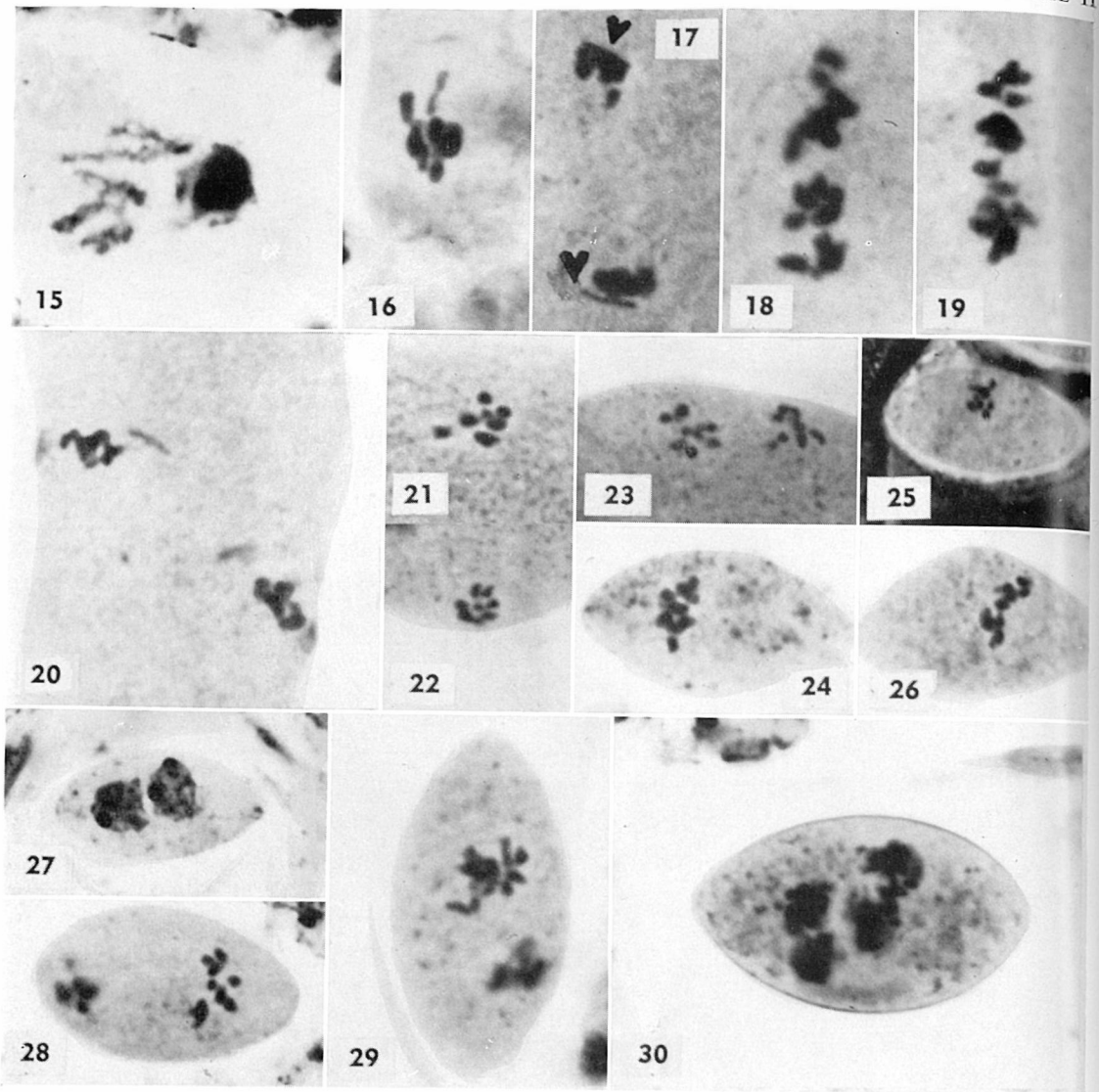

FIGS. 15–30. *Poronia punctata*. 15. Diplotene. Note the large size of nucleolus, i.e., about the size seen at pachytene. 16. Metaphase I. Seven bivalents. 17. Telophase I. Note centrosomal plaques (arrows). 18, 19. Anaphase II. Both figures from the same ascus. Not all chromosomes have undergone disjunction of chromatids. 20. Anaphase II. Note centrosomal plaques. 21. Telophase II – metaphase III. Seven chromosomes. 22, 23. Telophase III. Seven chromosomes in each figure. 24, 25. Metaphase IV within ascospore. Seven chromosomes in each figure. 26. Early anaphase IV. 27. Binucleate ascospore following division IV. Interphase. 28, 29. Metaphase V. 30. Maturing quadrinucleate ascospore. Magnifications: Figs. 25, 30, ca. × 1500; Figs. 27, 28, ca. × 1800; Figs. 15, 29, ca. × 2000; Figs. 16, 20, 24, 26, ca. × 2200; Fig. 17, ca. × 2500; Figs. 18, 19, 21, 22, 23, ca. × 3000.

5p often appeared to be two associated chromosomes. Study of many preparations convinced me that chromosome 5 is indeed a single chromosome, probably with a somewhat submedian centromere. It is apparently quite susceptible to breakage during cytological preparation.

Very few diplotene–diakinesis stages were seen; Fig. 15 represents a typical diplotene configuration. Several meiotic metaphase I figures were observed. Usually, seven bivalents could be counted (Fig. 16). What have been called centrioles or centrosomes in Ascomycetes (10) are herein called centrosomal plaques on advice of D. von Wettstein, Carlsberg Laboratory, Copenhagen (personal communication); there is considerable question about their structure. At anaphase two centrosomal plaques are associated with a nucleus (Fig. 20); one centrosomal plaque is associated with each telophase nucleus (Fig. 17). Their relationship to the nuclear membrane could not be ascertained because membranes are seldom seen in material prepared and examined as that depicted here. Figures of meiotic division II were often encountered. Metaphase–anaphase figures usually showed unsynchronized disjunction of chromatids (Figs. 18, 19). Following telophase of division II the chromosomes remain more or less contracted, apparently because mitotic division III ensues immediately; telophase II and metaphase III are usually almost indistinguishable (Fig. 21). Many excellent figures of telophase III were encountered; the chromosomes usually could be counted (Figs. 22, 23). Following telophase III the chromosomes become elongated and diffuse, entering interphase.

Each of the eight nuclei is incorporated into an ascospore. Soon after ascosporogenesis, another mitosis (division IV) occurs (Figs. 24–26); ascospores become binucleate (Fig. 27). Shortly thereafter, another mitosis (division V) ensues (Figs. 28, 29). Maturing ascospores are quadrinucleate (Fig. 30).

Discussion

Poronia oedipus and *P. punctata* show haploid chromosome numbers of 7. Other investigated xylariaceous fungi obviously related to *Poronia* have the following haploid numbers: *Rosellinia limoniispora* Ellis and Everh., 6 (6); *Hypoxylon* species, 4 and 5 (see 14 for references); *Xylaria* species, 8 or 9 and 8 (12, 13). Haploid chromosome numbers of 7 are commonly encountered in sordariaceous fungi, e.g., *Neurospora* (15), *Sordaria* (2), *Podospora* (1).

The resemblance of *Poronia* to *Xylaria* and the difficulties in separating these genera at a certain point were discussed earlier. Difficulties may arise when the chief criteria for separation are gross stromatal morphology and substrate. When undoubted species of *Xylaria* and undoubted species of *Poronia* are compared, they show conspicuous differences in addition to stromatal and substrate characteristics. *Xylaria curta* Fr. and *X. polymorpha* (Pers. ex St. Amans) Grev. feature rather narrow ascospores that are uninucleate at maturity owing to loss of mitotic products in young ascospores and before ascosporogenesis, respectively (12, 13). *Poronia oedipus* and *P. punctata*, on the other hand, show broad ascospores that are binucleate and quadrinucleate, respectively, at maturity. Asci of *X. curta* and *X. polymorpha* are narrowly cylindrical; asci of *P. oedipus* and *P. punctata* are broad. As mentioned above, the chromosome numbers of investigated *Xylaria* species differ from those of investigated *Poronia* species. The conidial stage of *P. oedipus* (7) differs in several respects from conidial stages of investigated *Xylaria* species (see 5 for references); the conidial stage of *P. punctata* likewise differs, as will be discussed elsewhere. In any case, certain critical species that are difficult to assign either to *Xylaria* or to *Poronia* must be studied to determine if taxonomically useful differences are to be found. Moreover, species currently assigned to *Podosordaria* should be investigated to clarify their systematic position.

The resemblance of *Poronia* to some species of *Hypocopra* is striking, as recognized by Munk (9) and others. Unfortunately, *Hypocopra* has been studied in insufficient detail for me to draw conclusions here. It is also noteworthy that the *Poronia* species discussed herein have a suspiciously "sordariaceous" look, i.e., in the wide asci, broad ascospores, the haploid chromosome numbers, and the dung substrate.

Further studies of *Poronia* (sexuality, developmental morphology, and ultrastructure) are in progress.

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