

## Taxonomic Studies of Parasitic Nyctotherans from Chinese Anura Amphibians III. *Wichtermania*

Wu-Han Xiao, Jian-Guo Wang\* and Lian-Xiang Li

Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, China

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**Wu-Han Xiao, Jian-Guo Wang and Lian-Xiang Li (2002)** Taxonomic studies of parasitic nyctotherans from Chinese Anura amphibians III. *Wichtermania*. *Zoological Studies* 41(1): 69-76. This paper describes 6 new species belonging to the genus *Wichtermania* from Anura amphibians distributed in southern China. The 6 species are *Wichtermania multigranularis* sp. nov., *W. oviformis* sp. nov., *W. vesiformis* sp. nov., *W. reticulatis* sp. nov., *W. granuliformis* sp. nov., and *W. obliquoides* sp. nov. The characteristics for diagnosis of the 6 new species are described in this paper. <http://www.sinica.edu.tw/zool/zoolstud/41.1/69.pdf>

**Key words:** Anura amphibian, *Wichtermania*, Taxonomy, New species.

It is well known that there are many parasitic ciliated protozoa living in the posterior intestines of anuran amphibians. Some of them such as opalinids or nyctotherans are almost all parasitic living in anuran amphibians. But whether these ciliates are harmful or helpful to their hosts is still unknown. There are many reports about these parasitic nyctotheran ciliates in amphibians. Especially in India and other Asian countries, more than 100 species have been described. Earl (1972) according to a species, *Nyctotherus cheni* Wichterman, 1934, established new genus *Wichtermania* in 1972. Since, there have been few reports about the genus. In 1990, we began to investigate parasitic nyctotherans from anuran amphibians distributed in southern China (Li et al. 1998, Wang et al. 1998, Li et al. 2002). Through examining 13 species belonging to anuran amphibians, six species of *Wichtermania* were found to be new records to China. The characteristics for diagnosis of the 6 new species are reported herein.

### MATERIALS AND METHODS

Specimens of the host were directly captured in the wild or were bought from different local markets in southern China. The parasites were collected and fixed in 5% formalin. In addition to immersing them in 10% glycerine-alcohol, several specimens of parasites were also stained using hematoxylin and eosin (H & E), after being fixed with Schaudinn's solution.

In order to observe the morphology of the macronucleus, micronucleus, and cytopharynx tube, the method of immersing in polyvinyl alcohol was also employed. Otherwise, Klein's dry silver method was also used to observe the sutural line and the arrangement of the cilia.

All specimens are deposited at the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China.

### DIAGNOSIS

#### *Wichtermania multigranularis* sp. nov.

(Figs. 1, 2)

Host and infection site: Rectum of *Rana spinosa*.

Locality and distribution: China: Lichuan Co., Hubei Prov.;

\* To whom correspondence and reprint requests should be addressed. Tel: 86-27-87647720/87647721. Fax: 86-27-87647679. E-mail: wangjg@ihb.ac.cn

Lingchuan Co., Guangxi Prov.

Body oval, pointed at anterior end and bluntly rounded at posterior end, consisting of upper and bottom flaps, left surface relatively convex, slightly concave on right surface, flatter in front and thicker at posterior part. Upper flap covering bottom flap like 2 different-sized ciliates overlapping together. In front of left surface with a distinct "λ" shaped sutural line, ciliary rows of left interior side arciform, with several parallel ciliary rows on left side, also with a naked region having no cilia. A distinct sutural line on right surface "λ" shaped, parallel ciliary rows on exterior of right side originating from this sutural line to posterior part, arciform. Oral groove elongated from anterior end of body occupying about 1/2 body length. Adoral zone of membranelle (AZM) distinct from tip of oral groove and extending from wall of cytopharynx to posterior end. Cytopharynx "J" shaped, bent very much at terminal end forming an angle of about 140° with longitudinal axis of body, terminal end of cytopharynx in oblique line, micronucleus unknown. A single contractile vacuole near anal canal as usual. Cytoplasm abundant, uniform and containing many different-sized granules, the largest 10 μm in maximum diameter. Upper flap

189.0 (164.7-224.1) μm long, 127.8 (108.0-143.1) μm wide; bottom flap 209.0 (191.7-224.0) μm long, 140.9 (124.2-151.2) μm wide; oral groove 90.7 (83.7-105.3) μm long; cytopharynx 120 (120.6-127.6) μm long and 23.8 (21.6-29.7) μm wide, and 35.1-45.9 μm in interior diameter of bend of cytopharynx. Macronucleus 44.9 (35.1-51.3) μm long and 31.7 (27.0-37.8) μm wide.

The outline, arrangement of ciliary rows, and the form of the cytopharynx of this new species are similar to those of *Wichtermania* (*N.*) *cheni* Earl, 1972, but the body of this new species is considerably larger, possesses asymmetric ciliary rows, and contains many big granules in the cytoplasm.

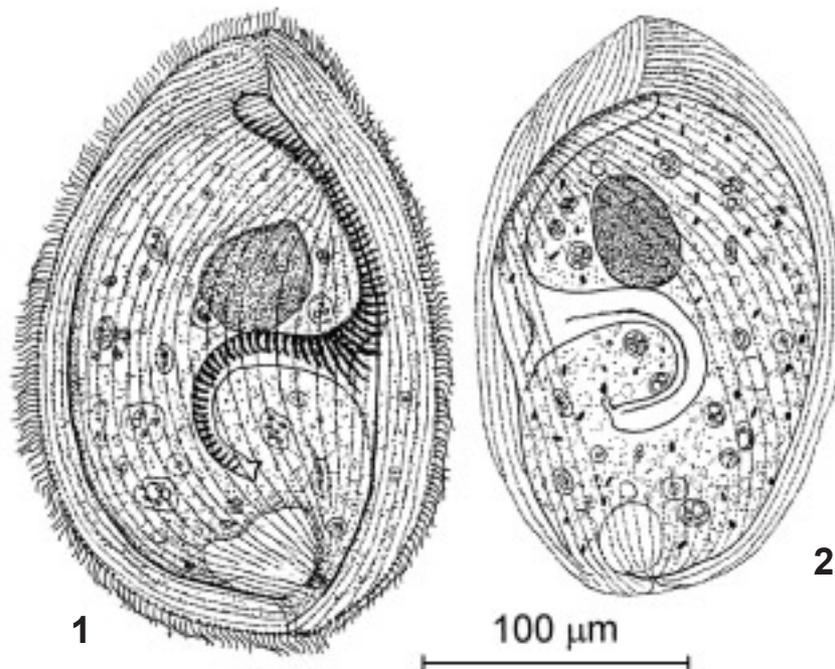
***Wichtermania oviformis* sp. nov.**

(Figs. 3, 4, 5, 6)

Host and infection site: Rectum of *Rana spinosa* and *R. plauraden*.

Locality and distribution: China: Guilin City and Lingchuan Co., Guangxi Prov.

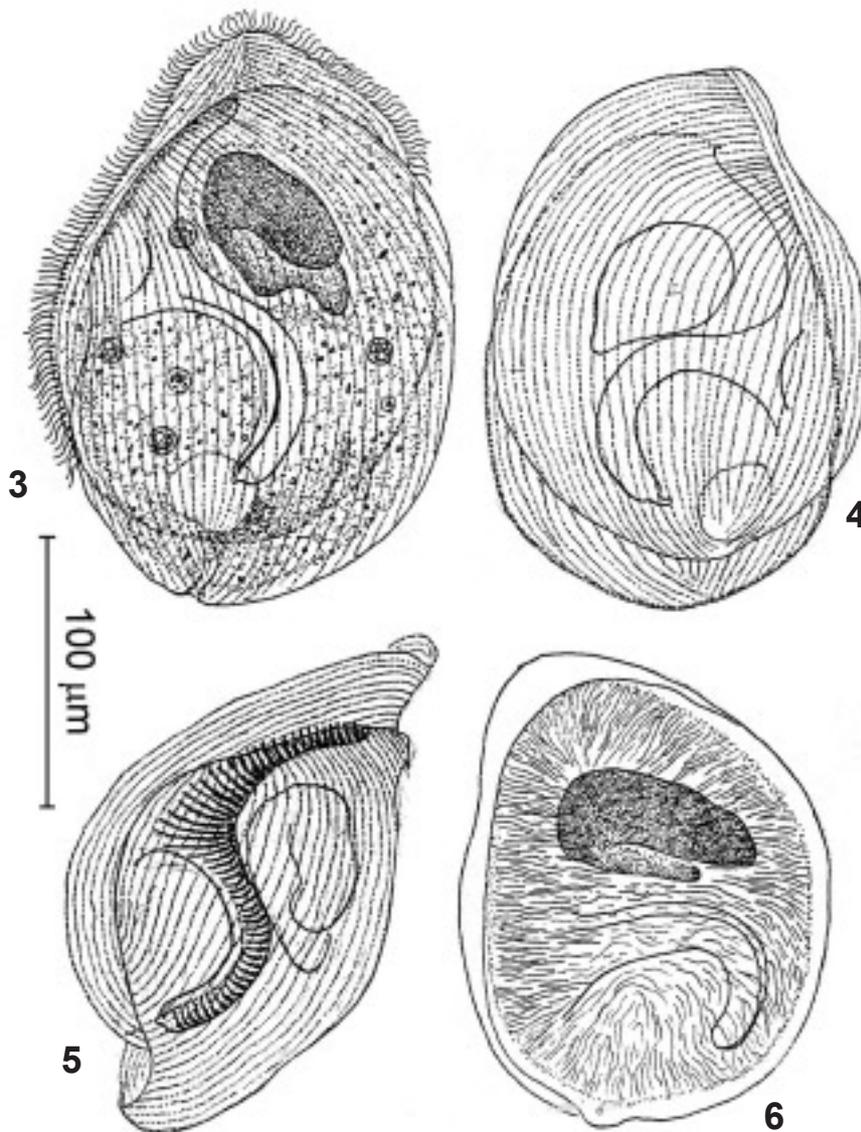
Body oval, relatively transparent, thin at anterior end and thicker at posterior part, consisting of distinct upper and bottom flaps with upper flap covering bottom flap, and bottom flap the same as



**Figs. 1-2.** *Wichtermania multigranularis* sp. nov. 1. Right surface view, showing shape of the body, ciliary row, sutural line, macronucleus, and cytopharynx large granules in cytoplasm; 2. Left surface view, showing sutural line, ciliary row, macronucleus, cytopharynx, cytopharyngeal canal, and cytoplasmic inclusions. Specimens fixed in 5% formalin and immersed in 10% glycerine-alcohol (glycerine 10 ml + 70% alcohol 90 ml).

an abdominal foot of a snail. Left surface convex, flat or slightly concave on right surface, front of left surface with a "λ" shaped sutural line, with a sutural line on right surface also. Ciliary rows uniform, without naked region, arrangement of ciliary rows as in *W. (N.) cheni* Earl, 1972. Oral groove distinct and occupying about 1/2 of body length. AZM from tip of oral groove running along outer wall of cytopharynx to terminal end. Cytopharynx "J" shaped, slightly extending at anterior side and bent at posterior part, forming an angle of about 120° with longitudinal axis of body. Macronucleus

sausage shaped, folding on tip of cytopharynx as usual. Micronucleus unknown, contractile vacuole and anal canal rift shaped. Cytoplasm abundant and containing a few big granules. A fine, striated, endoplasmic platelet under pellicle of left surface of body, front of body with centripetal fibers, with some concentric fibers at posterior part. Upper flap 175.7 (151.2-191.7) μm long, 109.3 (102.6-153.9) μm wide; bottom flap 182.6 (170.4-197.0) μm long, 143.2 (129.6-152.3) μm wide. Oral groove 70.9 (67.5-75.6) μm long; macronucleus 65.0 (45.9-81.0) μm long and 35.6 (27.0-43.0) μm



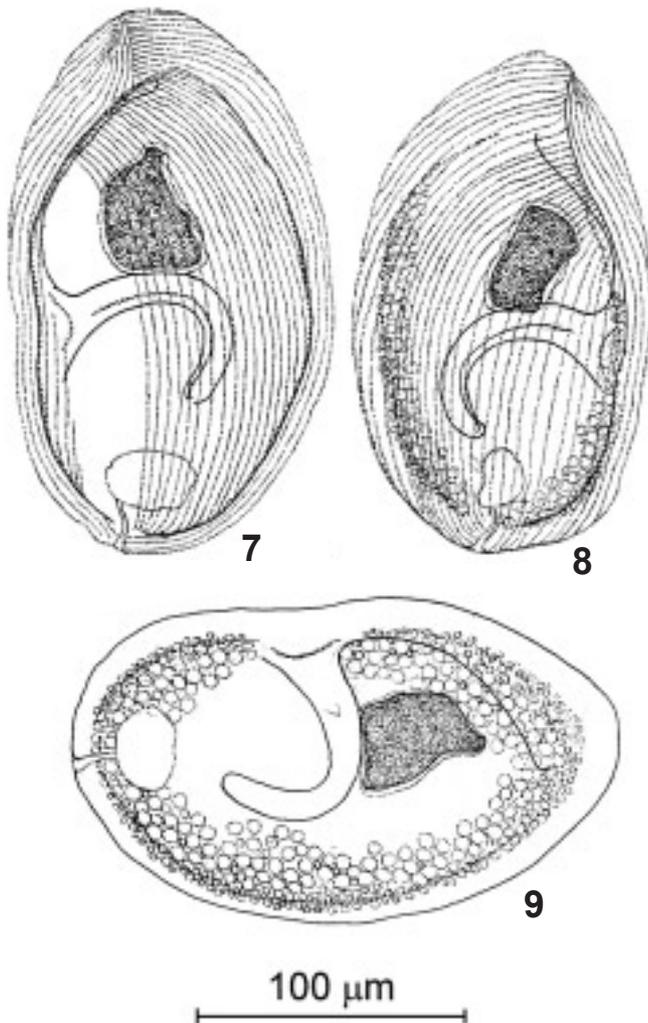
**Figs. 3-6.** *Wichtermania oviformis* sp. nov. 3. Left surface view, showing shape of the body, consisting of upper and bottom flaps, sutural line, and ciliary row, and the shape of the macronucleus, cytopharynx, and cytopharyngeal canal; 4. Right surface view, showing sutural line, and ciliary row; 5. Lateral view, showing upper and bottom flaps; 6. Left surface view, showing macronucleus, cytopharynx, and striated endoplasmic platelet.

wide.

The appearance, arrangement of ciliary rows and situation of the sutural line of this new species are similar to those of *W. reticulatis* sp. nov. and *W. vesiformis* sp. nov. This new species differs from them in having uniform ciliary rows, two distinct flaps, and a striated endoplasmic platelet. The endoplasmic platelet of this ciliate is also similar to that of *W. obliquoides* sp. nov., but the latter is larger than the former, the structure of the anterior side of the cytopharynx greatly differs, and it lacks a naked region.

***Wichtermania vesiformis* sp. nov.**

(Figs. 7, 8, 9)



**Figs. 7-9.** *Wichtermania vesiformis* sp. nov. 7. Left surface view, showing sutural line, ciliary row, macronucleus, and naked region; 8. Right surface view, showing sutural line, ciliary row, macronucleus, cytopharynx, and vesicular cytoplasm; 9. Left surface view, showing macronucleus, cytopharynx, and vesicular cytoplasm.

Host and infection site: Rectum of *Rana plauradon*.

Locality and distribution: China: Lichuan Co., Hubei Prov.; Lingchuan Co., Guangxi Prov.

Body oval or nearly elliptical, pointed at anterior end, rounded bluntly at posterior end and with distinct upper and bottom flaps. Arrangement of ciliary rows and form of sutural line the same as those of *W. (N.) cheni* and *W. oviformis* sp. nov. Oral groove elongate, occupying about 1/3 to 1/2 of body length. AZM distinct, cytopharynx "J" shaped, forming an angle of about 140° with longitudinal axis of body, without cytopharynx canal. Macronucleus nearly square, sitting on tip of cytopharynx in oblique line or at right angle, micronucleus unknown. Possessing a contractile vacuole and anal canal, but lacking on endoplasmic platelet under the pellicle. Cytoplasm very abundant, with many small, vesicular granules around periphery of body, but a few in central portion. Upper flap 181.5 (156.6-194.4) μm long, 114.0 (94.5-126.9) μm wide; bottom flap 227.5 (189.4-243.0) μm long and 137.5 (118.8-154.8) μm wide. Oral groove 105.5 (97.2-110.7) μm long; cytopharynx 116.5 (105.3-143.1) μm long and 19.9 (18.9-21.6) μm wide. Macronucleus 43.3 (37.8-59.4) μm long and 27.8 (21.6-29.7) μm wide.

The appearance, distribution of the ciliary rows, and situation of the sutural line as well as bend angle of the cytopharynx of this new species are similar to those of *W. (N.) cheni*, *W. reticulatis* sp. nov., and *W. multigranularis* sp. nov., being especially similar to *W. multigranularis*, but differing from them in size and form of the macronucleus and by having many small vesicular granules on the periphery of the body.

***Wichtermania reticulatis* sp. nov.**

(Figs. 10, 11, 12)

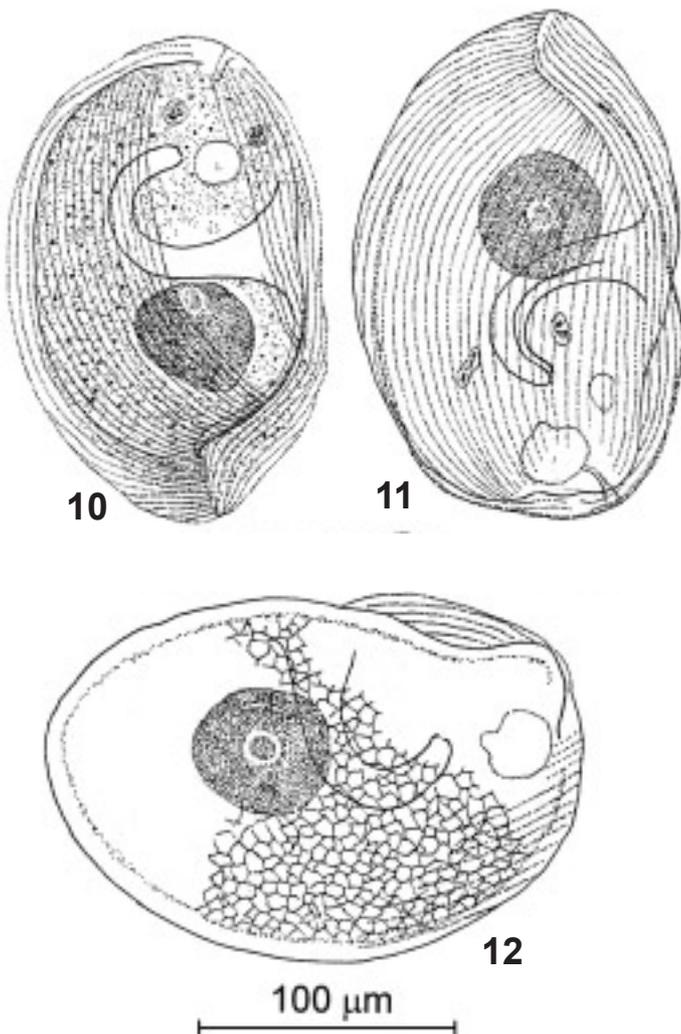
Host and infection site: Rectum of *Rana spinosa*.

Locality and distribution: China: Lingchuan Co., Guangxi Prov.

Outline of body, distribution of sutural line, and "J" shape of cytopharynx the same as those of *W. (N.) cheni*, *W. multigranularis*, and *W. vesiformis*, but differing in the round shape of the macronucleus and by having small, clear, uniform chromatin within. In addition to surface under the pellicle of the right having a reticular endoplasmic platelet, the terminal part of the cytopharynx forms an angle of about 140° with the longitudinal axis of the body. Both the contractile vacuole and anal canal are very clear. Upper flap 196.5 (153.9-237.6) μm long, 140.2 (102.6-170.1) μm wide; bot-

tom flap 226.4 (178.2-270.0)  $\mu\text{m}$  long and 152.3 (118.8-175.5)  $\mu\text{m}$  wide. Oral groove 92.1 (75.6-110.7)  $\mu\text{m}$  long; cytopharynx 115.2 (97.2-129.6)  $\mu\text{m}$  long and 22.2 (18.9-27.0)  $\mu\text{m}$  wide; macronucleus 54.6 (40.5-67.5)  $\mu\text{m}$  long and 46.2 (32.4-56.7)  $\mu\text{m}$  wide.

The outline of the body, distribution of ciliary rows, sutural line, and angle of the bend of the cytopharynx of this new species are similar to those of *W. (N.) cheni*, and *W. vesiformis* sp. nov., but this species differs from them in the large size of the body, rounded macronucleus, and in having a reticular endoplasmic platelet under the pellicle.



**Figs. 10-12.** *Wichtermania reticulatis* sp. nov. 10. Left surface view, showing shape of the body, sutural line, ciliary row, naked region, macronucleus, and cytopharynx; 11. Right surface view, showing shape of the body, macronucleus, clear region, cytopharynx, and reticular structures; 12. Right surface view, showing shape of the body, sutural line, ciliary row, macronucleus, clear region, and cytopharynx.

***Wichtermania granuliformis* sp. nov.**

(Figs. 13, 14, 15)

Host and infection site: Rectum of *Rana spinosa*.

Locality and distribution: China: Suburb of Guilin City, Guangxi Prov.

Body oval, consisting of upper and bottom flaps, upper flap convex, covering bottom flap, bottom flap relatively large with a distinct margin. Left surface slightly convex, right surface flat or slightly concave and thin at anterior end, but thick at posterior part. Sutural line " $\lambda$ " shaped, parallel ciliary rows on out side of left side, while all ciliary rows on interior of left side originating from this sutural line and running toward posterior part, arciform, in middle portion of left surface with notable naked region. Sutural line also on right surface, ciliary rows arciform on interior of right side, and with 8-10 parallel ciliary rows on outer part of right side. Oral groove relatively long from anterior end and occupying about 1/2 of body length. AZM conspicuous, from tip of oral groove running along outer wall of cytopharynx to posterior end of cytopharynx. Cytopharynx "J" shaped, extending funnel-like on anterior side and slightly narrowing, terminal end bent forming an angle of about  $160^{\circ}$ - $170^{\circ}$  with longitudinal axis of body. Cytopharyngeal canal indistinct. Macronucleus sausage or ribbon shaped, nuclear material uniform, without a clear region, forming an angle of about  $45^{\circ}$  intersecting with cytopharynx. With a single contractile vacuole, cytophyge rift-like. Cytoplasm abundant with many similar spherical granules. Upper flap 232.6 (229.0-243.0)  $\mu\text{m}$  long, 181.9 (167.0-192.0)  $\mu\text{m}$  wide; bottom flap 273.3 (265.0-281.0)  $\mu\text{m}$  long and 200.0 (186.0-213.0)  $\mu\text{m}$  wide, and 118.0-121.0  $\mu\text{m}$  thick. Oral groove 147.9 (135.0-175.5)  $\mu\text{m}$  long; cytopharynx 164.3 (129.6-189.0)  $\mu\text{m}$  long and 26.2 (21.6-29.7)  $\mu\text{m}$  wide; bent part of cytopharynx 45.5 (40.5-51.3)  $\mu\text{m}$  in inner diameter; macronucleus 62.6 (54.0-72.9)  $\mu\text{m}$  long and 37.5 (36.5-56.3)  $\mu\text{m}$  wide.

The shape, arrangement of ciliary rows, situation of sutural line, and bend of the cytopharynx of this new ciliate is similar to those of *W. vesiformis* sp. nov. and *W. (N.) cheni*, being especially similar to *W. vesiformis*, but this species differs in its large size, longer oral groove, larger angle of the cytopharynx, and by the existence of solid granules in the cytoplasm.

***Wichtermania obliquoides* sp. nov.**

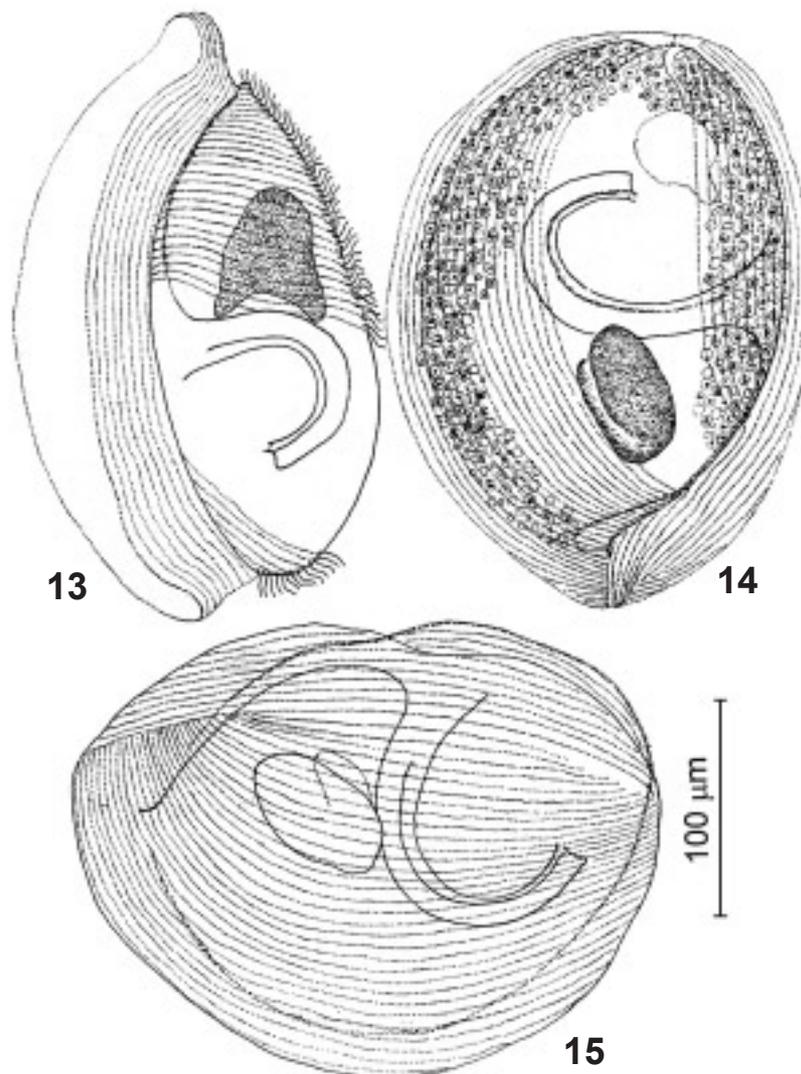
(Figs. 16, 17, 18, 19)

Host and infection site: Rectum of *Rana spinosa*.

Locality and distribution: China: Lingchuan Co. and Guilin City, Guangxi Prov.; Lichuan Co., Hubei Prov.

Body ellipsoid, pointed at anterior end, left surface convex, right surface flat or slightly concave, thick in middle portion, upper and bottom flaps distinct, sutural lines on left and right surfaces conspicuous, arrangement of ciliary rows the same as that of *W. (N.) cheni* and *W. granuliformis* sp. nov., also with a naked region on left surface. Oral groove running along outer wall of cytopharynx to terminal end. Cytopharynx "J" shaped, narrower at anterior part, continuing up forming an angle of about 140°-145° with longitudinal axis of body. Macronucleus folded both ends

together usually, ellipsoid or oval, and on top side of oblique line, micronucleus unknown. With distinct contractile vacuole or several vesicles near end of cytopharynx, cytophyge rift-like. Cytoplasm abundant and containing much bacteria and a few granules, with a striated endoplasmic platelet under the pellicle of right surface. Upper flap 201.1 (162.0-243.0)  $\mu\text{m}$  long, 174.5 (168.0-186.3)  $\mu\text{m}$  wide; bottom flap 238.6 (159.7-305.1)  $\mu\text{m}$  long, 171.5 (135.0-199.0)  $\mu\text{m}$  wide; oral groove 104.8 (81.0-151.2)  $\mu\text{m}$  long; cytopharynx 129.69 (121.5-143.0)  $\mu\text{m}$  long, 19.5 (17.5-21.6)  $\mu\text{m}$  wide and its narrow part 13.5  $\mu\text{m}$  wide; 51.6 (40.5-64.8)  $\mu\text{m}$  in outside diameter of bend of cytopharynx.



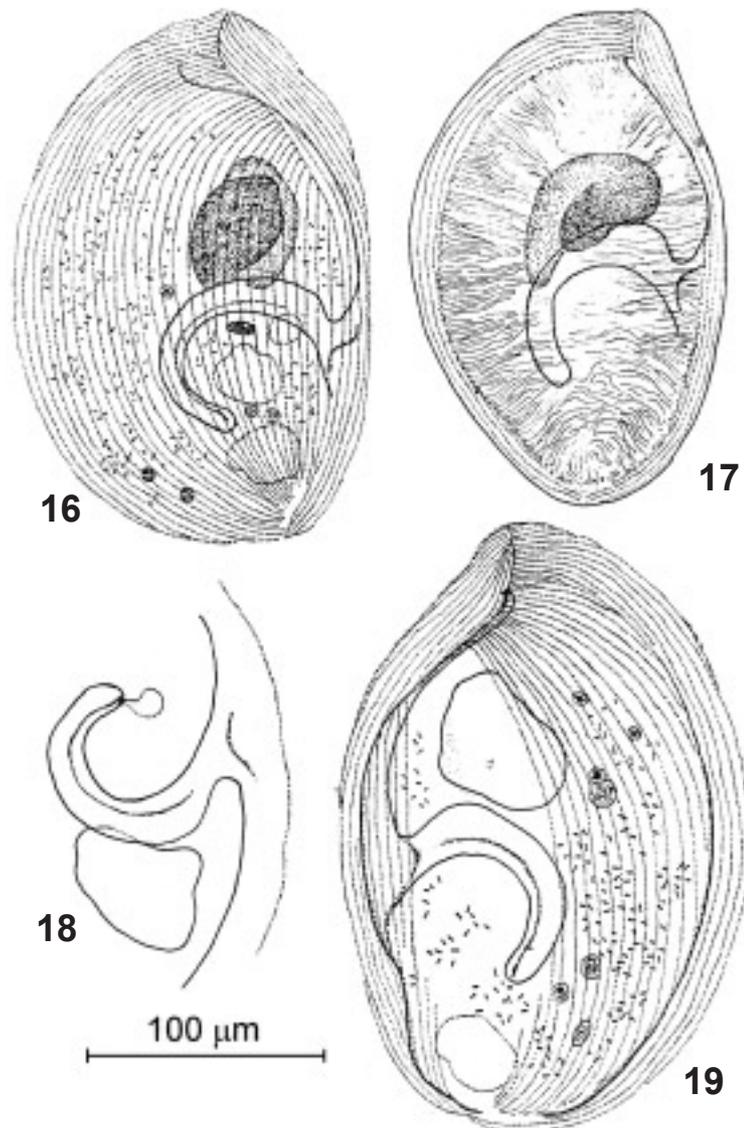
**Figs. 13-15.** *Wichtermania granuliformis* sp. nov. 13. Right surface view, showing shape of the body, the sutural line, ciliary row, naked region, shape of the macronucleus and cytopharynx, and granules in the cytoplasm; 14. Lateral view, showing upper and bottom flaps, ciliary row, naked region, macronucleus, and cytopharynx; 15. Right surface view, showing sutural line, ciliary row, macronucleus, and cytopharynx.

The shape, arrangement of ciliary rows, and sutural line of this new species are similar to those of *W. (N.) cheni* and *W. vesiformis* sp. nov., but the latter 2 species have no striated endoplasmic platelet. The striated endoplasmic platelet is also similar to that of *W. oviformis* sp. nov., but the shape of the cytopharynx and the body form of *W. oviformis* are greatly differ.

## DISCUSSION

Earl (1972) according to the species *Nyctotherus cheni* Wichterman, 1934 established

the new genus *Wichtermania*. The characteristics of this genus are that the ciliated body surface is composed of 2 flaps with 2 short sutural lines on the anterior end of its body, the cytopharynx is "J" shaped, and the terminal end greatly bends forming an angle of 120°-180°. The body characteristics are similar to those of *Nyctotheroides* (Grassé 1926) Corliss, 1969, *Parasicuophora* Albaret 1968, *Nyctotherus* Leidy, 1849, and *Pseudonyctotherus* Earl, 1970, especially to those of *Nyctotheroides* and *Nyctotherus*, but *Nyctotheroides* is not divided into 2 flaps, does not have a sutural line on the surface of the body, and the bend of the terminal end of the cytopharynx is less than 90°.



**Figs. 16-19.** *Wichtermania obliquoids* sp. nov. 16. Left surface view, showing shape of the body, sutural line, ciliary row, naked region, macronucleus, and shape of the cytopharynx; 17. Right surface view, showing sutural line, ciliary row, macronucleus, and cytopharynx; 18. Showing shape of the macronucleus and cytopharynx; 19. Right surface view, showing sutural line, macronucleus, cytopharynx, and the striated structure under the pellicle.

*Parasacuophora* has only 1 sutural line on the left side of the body and has the same parallel ciliary rows on the right side of the sutural line. Most species of this genus possess a single stiff bristle on the bottom of the exterior side of the cytopharynx. Species of *Nyctotherus* are also not divided into 2 flaps, lack a sutural line, and the bend of the terminal end of the cytopharynx is less than 90°. Species of *Pseudonyctotherus* differ from those of *Wichtermania* by the absence of a contractile vacuolum. We quite agree with Earl's ideas and think that the above characteristics of the genus *Wichtermania* are not only important on the basis of examined species, but also for the considerable differentiation of both genera of these ciliates. Based on this idea, we add 6 species as new members within *Wichtermania*. It is a good reminder that the original specimens of *Wichtermania* (*Nyctotherus cheni* Wichterman, 1934) Earl, 1972 were discovered by Dr. T.H. Chen from a frog, *Rana spinosa*, at Fukein Christian University, Fukein, China in 1934. We have found the same frogs in our investigation in wild fields (Lichuan Co., Hubei Prov.; Guilin City, and Lingchuan Co., Guangxi Prov.) and found more than 6 species of *Wichtermania*. So we think that this frog, *Rana spinosa*, can be found here and that these ciliates may be found in the intestine of this frog throughout its range.

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## 中國無尾兩棲類寄生腸腎蟲的分類研究 III. 韋氏腸腎蟲屬 (*Wichtermania*) 六新種的描述

肖武漢 汪建國 李連祥

本文描述了寄生無尾兩棲類的韋氏腸腎蟲屬 (*Wichtermania*) 的六個新種，它們是多粒韋氏腸腎蟲 (*Wichtermania multigranularis* sp. nov.)，卵形韋氏腸腎蟲 (*Wichtermania oviformis* sp. nov.)，泡狀韋氏腸腎蟲 (*Wichtermania vesiformis* sp. nov.)，網狀韋氏腸腎蟲 (*Wichtermania reticulatis* sp. nov.)，粒狀韋氏腸腎蟲 (*Wichtermania granuliformis* sp. nov.) 和傾斜韋氏腸腎蟲 (*Wichtermania obliquoides* sp. nov.)。

**關鍵詞：**無尾兩棲類，韋氏腸腎蟲屬，分類，新種。