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Организация ресничных и фибриллярных структур инфузо-рии *Bursaria truncatella* и ее систематическое положение [1]

Ciliary and Fibrillar Structures of the Ciliate *Bursaria truncatella* and its Systematic Position [1]

Синопсис. Проведено изучение ресничных и фибриллярных структур инфузории *Bursaria truncatella*. Исследование показало, что по строению кортекса, в том числе по организации эктоплазматической и околоротовой фибриллярных систем, а также по ряду структурных особенностей *B. truncatella* принципиально отли-чается от инфузорий из отр. *Heterotrichida* и вообще от простейших из п/кл. *Spirotricha*.

По целому ряду морфологических признаков *Bursaria* должна быть пере-несена (согласно системе — Corliss 1975) в кл. *Kinetofragminophora* п/кл. *Vestibulifera* и выделена в особый отряд *Bursaridida*, родственный отряду *Colpodida*. Согласно нашей макросистеме (Серавин и Герасимова 1977) *B. truncatella* перемещается из п/кл. *Spirotricha* в п/кл. *Homotricha*.

В работе высказывается предположение о целесообразности ревизии систе-матики гетеротрих.

Использование электронного микроскопа для изучения тонкой органи-зации инфузорий за относительно короткий период времени позволило су-щественно углубить, а в некоторых случаях и изменить наши сведения и пред-ставления об этих простейших.

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[1] Материалы статьи доложены 27 февраля 1978 г. на заседании Всесоюзного Обще-ства протозоологов СССР.

Большого внимания, с нашей точки зрения, заслуживает изучение кортикальных фибриллярных систем инфузорий, образуемых волокнистыми дериватами кинетосом. Эти системы обладают определенным зволюционным консерватизмом и знание их строения позволяет выяснять филогенетические взаимоотношения между разными группами инфузорий и уточнять их систематическое положение (Grain 1969, Герасимова и Серавин 1976, Lynn 1976, Серавин и Герасимова 1977).

В данной работе рассматривается организация ресничных структур и тонкое строение кортикальных (соматических и околоротовых) фибриллярных систем инфузории *Bursaria truncatella*. Попутно отмечается строение некоторых органелл, морфологически связанных с этими системами. Полученные нами данные позволяют, на наш взгляд, поставить вопрос о пересмотре систематического положения этой весьма обычной инфузории пресных вод.

Материал и Методика

Инфузории *Bursaria truncatella* O. F. Müller культивировались по методу Соловьевой (1946) в модификации Сергеевой (1976, 1977). Для фиксации отбирались вегетативные особи примерно в середине интервала между двумя делениями.

Для светооптического изучения кортекса был использован метод серебрения по Шаттону и Львову в модификации Corliss (1953).

Для электронномикроскопического исследования клетки *B. truncatella* фиксировались при 4°C 1% раствором OsO_4, приготовленным на ацетат-вероналовом буфере (pH 7.2) с добавлением сахарозы (7.5% сахарозы в фиксаторе) в течение 1 часа. Необходимость введения в фиксирующий раствор 7.5% сахарозы была обоснована ранее (Сергеева 1977). Далее объекты промывались ночь в 7.5% растворе сахарозы на ацетат-вероналовом буфере (pH 7.2) и затем обезвоживались в этиловых спиртах (50%, 70%, 90%, 100%), причем, для разведения спиртов вместо воды использовался 50% раствор NaCl и в 70% спирте, насыщенном уранил-ацетатом, клетки оставлялись на ночь. Заливка производилась в аралдит. При переносе клеток из спирта в аралдит они выдерживались 5 минут в эфире и затем в смесях эфира с аралдитом (1:1, 1:3, 1:7). Срезы на сетках контрастировались цитратом свинца по Fiske (1966) и изучались в электронных микроскопах Hitachi HU IIE и JEM 100°C.

Результаты

Светооптические наблюдения (Рис. 1, Табл. I, 1–4)

Обычная длина *Bursaria truncatella* — 0.6–0.8 mm, однако это простейшее может достигать длины до 2 mm и тогда его легко можно разглядеть невооруженным глазом. Инфузория имеет форму мешка (бурсы), широко откры-
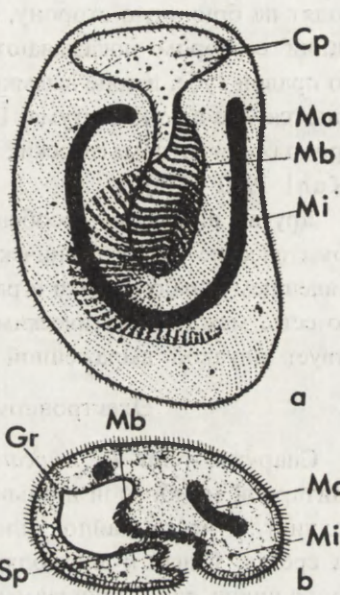
Рис. 1. *Bursaria truncatella* O. F. Müll. a — общий вид с брюшной стороны. b — на поперечном срезе; Ma — макронуклеус, Mi — микронуклеус, Mb — мембранеллы, Gr — щель, Cp — поры сократительных вакуолей, Sp — септум

Fig. 1. *Bursaria truncatella* O. F. Müll, a — general view, ventrally, b — transverse section; Ma — macronucleus, Mi — micronucleus, Mb — membranelles, Gr — furrow, Cp — pulsating vacuole pores, Sp — septum

того спереди. Внутренная полость мешка образует глубоко идущую почти до самого заднего конца тела околоротовую воронку, которую принято называть перистомальной; она имеет вид изогнутого слева направо рога. Бурса (воронка) этой инфузории на брюшной стороне рассечена глубоким разрезом-выемкой, доходящей до задней трети тела, так что образуются две протоплазматические губы (левая и правая).

На поперечных срезах через тело *Bursaria* хорошо видно, что воронка несет два внутренних выступа или выроста, идущих от самого переднего края ее до самой узкой ее части.

Слева на переднем конце воронки начинается широкий выступ, на котором расположены мембранеллы. Сначала этот выступ идет почти вертикально вниз, затем круто заворачивает направо по спинной стороне бурсы, затем снова идет вниз и, наконец, поворачивает к месту, где располагается цитостом инфузории.

Второй выступ воронки лежит справа, он узок, имеет лентовидную форму и несет ряды ресничек. Это септум *Bursaria*.

В правой части бурсы имеется также продольное впячивание — щель, которую некоторые авторы считают „ротовой щелью". Однако настоящий цитостом находится на дне бурсы и истинное физиологическое значение щели остается невыясненным.

Поверхность тела *Bursaria truncatella* покрыта тесно сближенными рядами ресничек. Кинеты идут не строго вдоль тела. Так, например, на спинной стороне они загибаются, начиная от заднего конца тела, справа налево и пере-

ходят на брюшную сторону, где тоже расположены косо. Результаты импрегнации серебром показывают, что соматические кинеты бурсарии, идущие
по правой губе, в зоне выемки делают крутой изгиб (почти под 90°) и продолжаются уже в зоне септума. Иначе говоря, ресничные ряды септума являются
продолжением соматических поверхностных кинет. Этот факт отмечал еще
(Kahl 1930–1935).

Другая интересная особенность этой инфузории заключается в том, что
соматические ряды ресничек, покрывающие левую губу *Bursaria*, подходя
к внешнему краю выемки и разворачиваясь, как бы переходят в мембранеллы.
То есть, между соматическими рядами и мембранеллами у *Bursaria* не существует никакой выраженной границы.

Электронномикроскопические наблюдения

Снаружи тело *B. truncatella* покрывает пелликула, которая образована
унитарной мембраной и альвеолярным слоем (Табл. II 5, IV 9–10). Альвеолы
пелликулы чрезвычайно уплощены, так что иногда их трудно различить
на срезах. Снизу к альвеолярному слою примыкает довольно значительный
по толщине слой эпиплазмы. В зонах кортекса, прилегающих к ресничкам
(циркумцилиарные депрессии), эпиплазма образует своеобразные фигуры:
она еще более утолщается и формирует валики, окружающие кольцом каждую
кинетосому. На поперечных срезах через кортекс эти кольцевые валики имеют
вид электронноплотных округлых телец, расположенных слева и справа от
кинетосомы (Табл. II 5). Под эпиплазмой у бурсарии расположена собственно
эктоплазма, содержащая обычный для инфузорий набор структур и органелл, среди которых довольно большой объем занимают мукополисахаридные включения (Табл. II 5, III 6, VI 13).

Соматические кинетосомы, входящие в состав ресничных рядов, в отличие
от большинства известных случаев, у *B. truncatella* собраны в триады (Табл.
III 6–7, IV 8). Две передние кинетосомы несут по ундулиподии, тогда как
задняя — лишена ее. Встречаются, хотя и редко, попарно расположенные
кинетосомы (диады); в этом случае обе они имеют ундулиподии.

Отдельные кинетосомы, входящие в состав триады, объединены электронноплотными связками в единый комплекс. Каждая триада кинетосом снабжена весьма своеобразным набором фибриллярных дериватов (Табл. III
6–7, IV 8, Рис. 2). У двух кинетосом триады — средней и задней — на уровне
девятого триплета отходит по постцилиарной фибрилле. Однако сильно
развита лишь та фибрилла, которая связана с задней кинетосомой. Другая
постцилиарная фибрилла триады имеет вид зачатка и не выходит за пределы своей кинетосомальной территории.

Постцилиарная фибрилла, отходящая от задней кинетосомы триады,
может состоять из 18–20 микротрубочек, которые расположены почти параллельно друг другу, так что образуется слегка изогнутая дугообразно ленто-

Рис. 2. Схематическое изображение триады соматических кинетосом с дериватами: у *Bursaria truncatella*. Pc—постцилиарные фибриллы, Kd — кинетодесмальный филамент, T — трансверсальная фибрилла

Fig. 2. Diagrammatic representation of a triade of somatic kinetosomes with their derivatives in *Bursaria truncatella*. Pc — postciliary fibrilles, Kd — kinetodesmal filament, T — transverse fibrilla
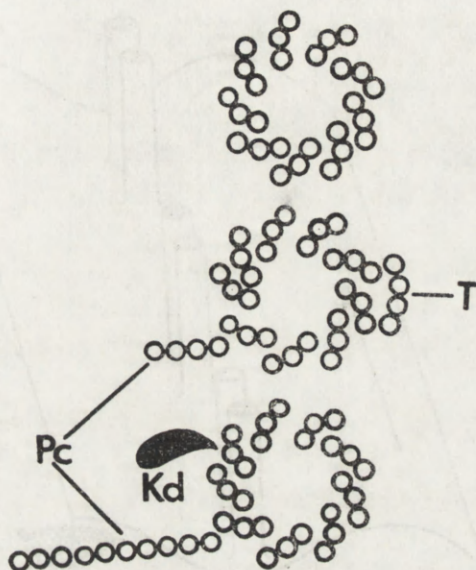
видная структура; отходя от своей триады, такая лента из микротрубоче- идет назад и вверх. Под пелликулой она налагается сверху на такие же постк- цилиарные фибриллы, идущие от впереди лежащих кинетосом того же рес, ничного ряда. Таким образом возникает постцилиодесма, которая представляет собой стопку постцилиарных фибрилл. Каждая постцилиодесма располагается в гребне пелликулы и занимает субпелликулярное положение. Состоит она обычно из 8–10 постцилиарных фибрилл. Это свидетельствует о том, что каждая такая фибрилла тянется вдоль 8–10 кинетодесмальных территорий. По мере удаления от исходной триады отдельная постцилиарная фибрилла постепенно перемещается к нижней стороне стопки фибрилл, становясь все тоньше и тоньше, пока не исчезает совсем (Табл. II 5, IV 9–10).

В каждой триаде от триплета № 7 задней кинетосомы берет начало короткий поперечно исчерченный кинетодесмальный филамент (Табл. III 6–7, IV 8).

Трансверсальная фибрилла возникает возле триплетов №3–№4 средней кинетосомы триады (Табл. III 6, 7, IV 8). Эта фибрилла очень коротка и далеко не всегда удается отыскать ее маленький фрагмент вблизи кинетосомы, дающей ей начало.

В отдельных редких случаях кинетосомы триад соматической зоны могут быть снабжены пакетами корневых нитей (Табл. IV 9).

Как уже упоминалось, иногда на фотографиях попадаются соматические кинетосомы, собранные в диады (Табл. III 6). Но и в этом случае трансверсальная фибрилла, микротрубочки которой видны тогда более отчетливо, кинетодесмальный филамент и две постцилиарные фибриллы (зачаточная и сильно развитая) сохраняют то же положение, что и в триаде.
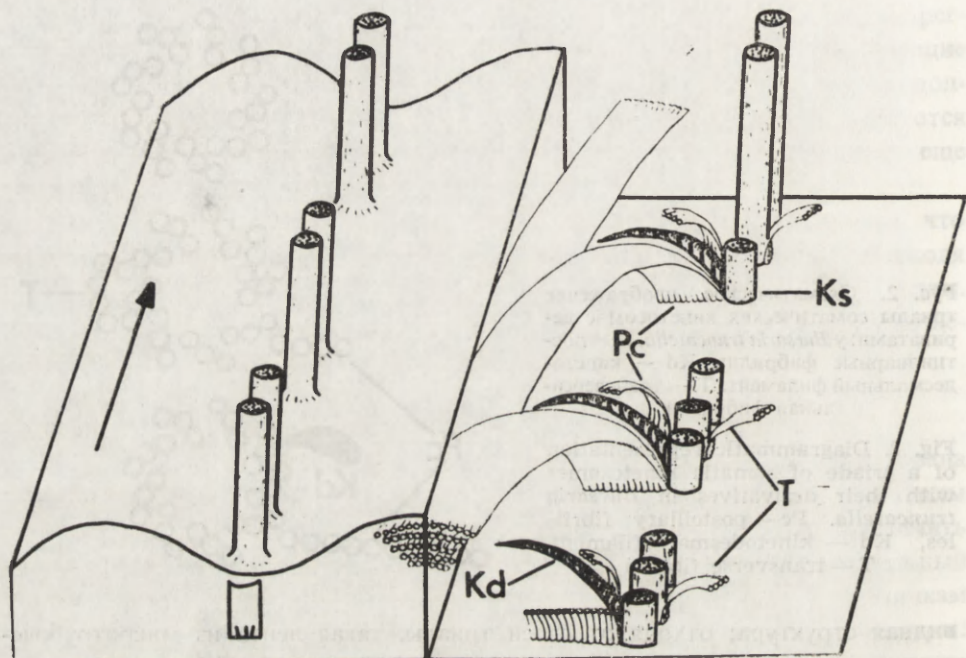
Рис. 3. Схематическое изображение организации эктофибриллярной системы у *Bursaria truncatella*. Расположенная справа стрелка указывает направление к переднему концу клетки, остальные обозначения как на Рис. 2

Fig. 3. Diagrammatic representation of ectofibrillar system organization in *Bursaria truncatella*. Oral end of the cell is indicated by an arrow, other explanations as in Fig. 2

На Рис. 3 изображена схема, показывающая организацию эктоплазматической фибриллярной системы *B. truncatella*.

Цилиатура перистомальной воронки у бурсарии, подобно соматической ее цилиатуре, имеет достаточно своеобразное строение. Мембранеллы адоральной зоны устроены следующим образом. Они состоят из триад, которые в пределах одной мембранеллы тесно сближены и развернуты по отношению к продольной оси этой органеллы под углом около 45° (Табл. VI 14, VIII 17). Каждая триада снабжена двумя ундулиподиями (Табл. V. 12), которые (как и в триадах соматической зоны) принадлежат двум передним кинетосомам. Фибриллярным дериватом каждой триады в мембранелле является коротенькая постцилиарная фибрилла, берущая начало от задней кинетосомы, лишенной ундулиподии (Табл. VI 14, VIII 17). Трансверсальных фибрилл мембранеллы *Bursaria* не имеют. Отсутствие трансверсальных фибрилл, связанных с буккальной цилиатурой, является чертой, мало характерной для инфузорий, имеющих постцилиодесмы (Серавин и Герасимова 1977). Это говорит о большом своеобразии строения околоротовой фибриллярной системы *B. truncatella*.

С проксимальной стороны кинетосомы мембранелл связаны с мощными ретикулярными структурами (Табл. V 11–12, VI 13, VII 15–16). Они образованы сетью микрофиламентов, которые соединяются друг с другом в определенных точках, называемых узлами конденсации. Эти ретикулярные структуры могут достигать огромных размеров и занимать в цитоплазме инфузории весьма большой объем. Повидимому, ретикулярная сеть армирует околоротовое пространство, позволяя ему сильно растягиваться без разрывов при заглатывании крупной добычи, а также сжиматься перед делением и инцистированием.

Септум перистомальной воронки снабжен ресничными образованиями, которые устроены совершенно иначе, чем мембранеллы адоральной зоны. (Табл. IX 18–19). Прежде всего следует отметить, что их кинетосомы также собраны в триады. Поскольку триады не сближены, то ряды, ими образуемые, весьма напоминают обыкновенные соматические кинеты. Сходство усиливается также тем, что кинетосомы этих триад (как и в соматической зоне бурсарии) снабжены парой ундулиподий. Кроме того, они имеют тот же набор волокнистых дериватов, что и соматические триады *B. truncatella*: две постцилиарные фибриллы, трансверсальную фибриллу и кинетодесмальный филамент. Правда, эти структуры развиты слабее, чем у соматических триад, но набор — тот же самый и столь же уникальный. А если вспомнить, что в световом микроскопе отчетливо видно, как соматические кинеты с поверхности бурсарии, разворачиваясь, переходят на внутреннюю сторону септума, то не остается никакого сомнения в том, что ресничные ряды септума являются частью соматической цилиатуры, заходящей в буккальную зону. Ретикулярные фибриллы у кинетосом ресничек септума отсутствуют.

Необходимо отметить, что пелликула перистомальной бурсы у *B. truncatella* представлена всегда лишь одной унитарной мембраной.

Нам не удалось обнаружить в перистомальной воронке бурсарии даже следов ундулирующей (пароральной) мембраны. Она отсутствует на любых продольных и поперечных срезах. Отсутствие этой органеллы у *Bursaria* заметил еще (Kahl 1930–1935).

Обсуждение

Со времен работы Perty (1852) по настоящее время *B. truncatella* относят к семейству *Bursariidae* Perty, которые включают в отряд *Heterotrichida* Stein подкласса *Spirotricha* Butschli класса *Polyhymenophora* [2] (Kahl 1930–1935, Kudo 1966, Corliss 1974, 1975, de Puytorac et al. 1974, Янковский 1975),

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[2] В пределах данной статьи мы придерживаемся системы инфузорий, предложенной Corliss (1975).

Однако по любой из ныне существующих систем к *Spirotricha* относятся такие инфузории, у которых на брюшной стороне имеется полихимениум, образованный рядом адоральных мембранелл и ундулирующей мембраной. Формула полихимениума такова $UM + M_1 + M_2 + \ldots + M_n$. Зона адоральных мембранелл (AZM) в этом случае расположена слева от рта и образует право-закрученную спираль; ундулирующая мембрана лежит справа от ротового отверстия. Соматические реснички у *Spirotricha* не заходят в перистомальное углубление. Также отсутствует у этих инфузорий связь между мембранеллами и соматическими рядами (в отличие от *Ciliata*, имеющих кинетофрагмон).

Уже светооптические наблюдения показывают, что *Bursaria* не является типичным представителем п/кл. *Spirotricha* отр. *Heterotrichida* в связи с тем что у нее: (1) нарушена формула полихимениума, так как *Bursaria* лишена ундулирующей мембраны; (2) соматические ряды ресничек заходят в бук-кальную зону — на септум; (3) наблюдается в зоне мембранелл тесная связь с соматической цилиатурой. Как видно из всего сказанного, у *Bursaria* рес-ничные образования, видимые в световом микроскопе, имеют организацию, значительно отличающуюся от организации тех же образований у *Spirotricha* (*Heterotrichida*).

Электронномикроскопические данные показывают точно так же, как и све-тооптические исследования, что по целому ряду признаков *Bursaria* принци-пиально отличается от типичных *Heterotrichida*, таких как *Spirostomum*, *Stentor*, *Blepharisma*, *Condylostoma*, *Climacostomum* и т.д. и от *Spirotricha* вообще.

У всех типичных *Heterotrichida* кинетосомы в соматических кинетах сдво-ены, каждая пара кинетосом несет только одну ундулиподию, одну пост-цилиарную и одну трансверсальную фибриллу; та и другая фибриллы сильно развиты (исключение составляет лишь *Stentor*, трансверсальные фибриллы которого развиты слабо) (Yagiu and Shigenaka 1963, Finley et al. 1964, Kennedy 1965, Grain 1968, Huang and Pitelka 1973, Peck et al. 1975 и т.д.). Каждая постцилиодесма у *Heterotrichida* занимает в кортексе латеральную позицию по отношению к кинете (Raikov et al. 1975) и образована стопкой постцилиарных фибрилл. Каждая постцилиарная фибрилла, отходя от своей кинетосомы, включается в основание такой стопки, то есть — снизу. По мере удаления от исходной кинетосомы, постцилиарная фибрилла постепенно утоньшаясь, перемещается к верхней части стопки и далее исчезает совсем. Схематически поперечный срез через такую латеральную постцилиодесму, характерную для типичных гетеротрих, можно представить в виде треуголь-ника, вершина которого направлена наружу к пелликуле (Рис. 4 a). Кинето-десмальный филамент у *Heterotrichida* полностью отсутствует (Grain 1968).

В отличие от *Heterotrichida* у *Bursaria* как уже отмечалось, соматические кинетосомы собраны в триады, которые несут две ундулиподии; трансвер-сальные фибриллы в данном случае развиты весьма слабо. Кроме того, в каж-дой триаде имеется кинетодесмальный филамент и вторая (зачаточная) пост-
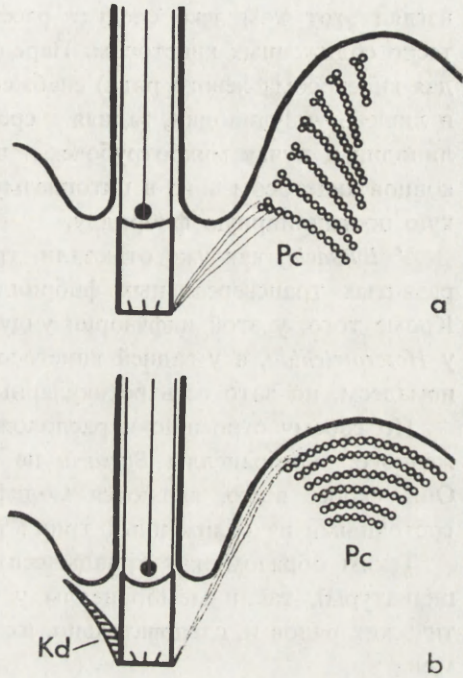
Рис. 4. Схематическое изображение организации и расположения постцилиодесмы у типичных *Spirotricha* (a) и у *Bursaria truncatella* (b). Обозначения те же, что на Рис. 2

Fig. 4. Diagrammatic representation of the structure and arrangement of the postciliodesm in typical *Spirotricha* (a) and in *Bursaria truncatella* (b). Explanations the same as in Fig. 2

цилиарная фибрилла. Постцилиодесмы *B. truncatella* также представлены стопками, но расположены они не латерально по отношению к кинете, а в середине гребня под пелликулой и организованы совершенно особым образом. Каждая постцилиарная фибрилла включается в такую стопку сверху и, по мере удаления от своей кинетосомы, постепенно перемещается к нижнему ее краю, уменьшаясь в размерах, пока не исчезнет совсем. Следовательно, схематический поперечный срез через субпелликулярную постцилиодесму у *Bursaria* будет иметь вид треугольника, вершина которого направлена внутрь клетки, а основание — наружу — к пелликуле (Рис. 4 b).

В настоящее время накопилось много доказательств того, что общий план строения эктоплазматической фибриллярной системы у инфузорий является весьма консервативным признаком (Герасимова и Серавин 1976, Lynn 1976, Серавин и Герасимова 1977). Поэтому наши данные служат веским аргументом при доказательстве отсутствия близкого родства *B. truncatella* с другими представителями отр. *Heterotrichida*. Тем более, что имеется еще много серьезных данных, говорящих о том, что *Bursaria* весьма отличается по своему строению от любых *Spirotricha*.

Буккальная цилиатура типичных *Heterotrichida* представлена, по данным французских авторов (de Puytorac и Grain 1976), большей частью мембранеллами, состоящими из трех продольных рядов кинетосом. На наш

взгляд этот комплекс следует рассматривать как образованный триадами тесно сближенных кинетосом. Передняя кинетосома отдельной триады (каждая кинетосома левого ряда) снабжена развитой трансверсальной фибриллой и лишена ундулиподии, задняя и средняя кинетосомы триады несут по ундулиподии и пучку микротрубочек — немадесм, отходящих от проксимальных концов кинетосом вниз в цитоплазму, задняя кинетосома также несет короткую постцилиарную фибриллу.

У *Bursaria*, как уже отмечали, триады мембранелл не только не имеют развитых трансверсальных фибрилл, но вообще лишены этих дериватов. Кроме того, у этой инфузории ундулиподия отсутствует не у передней, как у *Heterotrichida*, а у задней кинетосомы в триаде. Также нет у *B. truncatella* немадесм, но зато есть ретикулярные фибриллы.

По своему строению и расположению, как следует из всего выше изложенного, мембранеллы *Bursaria* не гомологичны мембранеллам *Spirotricha*. Они, скорее всего, являются модифицированными соматическими рядами, состоящими из сближенных триплетов этих рядов.

Таким образом, как триады септума (архаичные участки поверхностной цилиатуры), так и мембранеллы у *Bursaria* являются производными соматических рядов и, следовательно, их можно считать элементами кинетофрагмона.

Следует отметить, что мембранеллоподобные образования возникают у инфузорий независимо в самых различных группах и использование метода электронной микроскопии доказывает их негомологичность. Так, например, у *Nassula* (Tucker 1968, 1971) имеются мембранеллы, внешне составляющие полихимениум, однако все авторы относят эту инфузорию к кл. *Kinetofragminophora*. *Neobursaridium*, первоначально причисленный к отр. *Heterotrichida* (кл. *Polyhymenophora*) впоследствии был перенесен в отр. *Hymenostomatida* (кл. *Oligohymenophora*). Электронномикроскопические исследования показали (Nilsson 1969), что мембранеллы этой инфузории имеют развитые кинетодесмальные филаменты. Кроме того, в предротовой ее части были обнаружены пеникулюсы.

Отличается *Bursaria* от *Heterotrichida* и строением пелликулы в соматической зоне. Под унитарной мембраной у этой инфузории расположены крайне уплощенный альвеолярный слой и хорошо развитый слой эпиплазмы, тогда как у *Heterotrichida* нет ни уплощенных альвеол, ни эпиплазмы. Тело их покрывает простая унитарная мембрана.

В отличие от *Spirotricha* и *Heterotrichida*, имеющих одну или несколько пароральных мембран (Grain 1972), *B. truncatella* полностью лишена их.

Итак, по организации ресничного покрова, по тонкому строению кортекса, по организации эктоплазматической и околоротовой фибриллярных систем, а также по целому ряду других морфологических особенностей *B. truncatella* принципиально отличается от инфузорий, относящихся к отр.

Heterotrichida, да и вообще от простейших п/кл. *Spirotricha*. Это дает нам полное основание исключить *Bursaria* из состава этих систематических групп.

Тогда возникает вопрос, с какой же иной систематической группой инфузорий может быть связано это простейшее?

Погружение внутрь тела рта с образованием бурсы той или иной глубины наблюдается у инфузорий из кл. *Kinetofragminophora* п/кл. *Vestibulifera*. Для этих инфузорий характерно то, что в это впячивание (вестибулюм) заходят соматические ресничные ряды — элементы кинетофрагмона.

Как уже отмечалось ранее, так называемая воронка *B. truncatella* сохраняет часть первичной соматической цилиатуры, расположенной на септуме и соединяющейся с соматической цилиатурой поверхности тела. По другому выступу внутрь бурсы заходят так называемые мембранеллы. Эти ресничные структуры формируют у *B. truncatella* кинетофрагмон в бурсе, которую, в связи с этим можно считать вестибулюмом. Последний характерен для представителей п/кл. *Vestibulifera* (Corliss 1975). По-видимому, среди инфузорий этой группы и следует искать формы, филогенетически связанные с *Bursaria*.

Интересно отметить, что род *Balantidium* в свое время относимый к сем. *Bursariidae* отр. *Heterotrichida* (Kudo 1946), позднее был выделен в отр. *Trichostomatida* (Kudo 1966), принадлежащий теперь к п/кл. *Vestibulifera* (Corliss 1975).

Среди представителей инфузорий с вестибулюмом наибольшего предпочтения для сравнения заслуживают простейшие из отр. *Colpodida*, которые обнаруживают поистине поразительное сходство с *B. truncatella* в строении ряда структур. Об этом можно судить по работам, в которых изложены результаты электронномикроскопических исследований некоторых видов, принадлежащих к двум родам из отр. *Colpodida—Tillina* и *Colpoda* (Didier et Chossa 1972, Герасимова 1976, Lynn 1976 a, b, c, d).

Оказывается, что пелликула у *Bursaria*, *Tillina* и *Colpoda* устроена практически одинаково. Она образована унитарной мембраной, весьма сильно уплощенными альвеолами и подлежащим хорошо развитым слоем эпиплазмы. У всех трех инфузорий эпиплазма образует вокруг внутриклеточного основания ресничек утолщенные валики. Эктоплазма этих простейших также содержит весьма большое количество мукоидного секрета.

Соматические кинетосомы инфузорий из отр. *Colpodida* собраны в диады. Каждая диада (как и изредка попадающиеся диады *Bursaria*) снабжены парой ундулиподий. Как передняя, так и задняя кинетосомы диад колподид дают начало постцилиарной фибрилле; сильно развита лишь та, которая отходит от задней кинетосомы пары. Этот признак является уникальным и характерным только для *Bursaria* и *Colpodida*.

Постцилиодесмы *Colpodida* так же как и у *Bursaria* занимают субпелликулярное положение, хотя отдельные постцилиарные фибриллы и не собраны

в правильные стопки. Однако на ряде электроннограмм поперечных срезов кортекса *Colpoda* и *Tillina* можно уловить четкую тенденцию отдельных лент микротрубочек в постцилиодесме располагаться косо одна под другой, что весьма напоминает разъехавшуюся стопку лент (Герасимова 1976, Lynn 1976 a).

Трансверсальные фибриллы имеются и у *Bursaria* и у *Colpodida*, однако у последних они развиты несколько сильнее.

В диадах *Colpodida* найдены также слабо развитые кинетодесмальные филаменты, которые, как и у *Bursaria* расположены поперечно по отношению к оси диады.

Корневые нити, встречающиеся у соматических кинетосом *Bursaria*, отмечены также у *Colpodida*.

Суммируя все имеющиеся данные, можно с полной уверенностью утверждать, что *B. truncatella* должна быть исключена из отр. *Heterotrichida* п/кл. *Spirotricha* кл. *Polyhymenophora*.

Сходство строения пелликулы и эктоплазматической фибриллярной системы *Bursaria* с теми же структурами у *Colpodida* и наличие в ее "перистомальной воронке" элементов соматической цилиатуры показывает, что эта инфузория относится к п/кл. *Vestibulifera* кл. *Kinetofragminophora* (В макросистеме — Серавин и Герасимова 1977 — *Bursaria* переносится из п/кл. *Spirotricha* в п/кл. *Homotricha*, но остается в пределах кл. *Postciliodesmatophora*). Однако включить эту инфузорию в отр. *Colpodida* нельзя, так как имеется ряд серьезных оснований препятствующих этому. Безусловно, в процессе эволюции *B. truncatella* претерпела ряд морфологических изменений, которые отделяют ее от представителей этого отряда. Во-первых, соматическая цилиатура этой инфузории в основном построена из триад кинетосом (диады сохранились лишь в качестве рудиментов). Во-вторых, большая часть вестибулюма потеряла реснички (или их производные). В-третьих, часть сохранившейся цилиатуры вестибулюма бурсарии дала начало мембранеллоподобным органеллам. Эти и ряд других морфологических особенностей заставляют выделить *B. truncatella* в особый отряд *Bursaridida*. Следует ли к нему отнести инфузорий род *Thylacidium* и род *Bursaridium*, традиционно включаемых в сем. *Bursariidae* (Kahl 1930–1935, Kudo 1966) — покажут будущие электронномикроскопические исследования.

В настоящее время вообще есть серьезные основания полагать, что отряд *Heterotrichida* является сборным.

Помимо *Bursaria* можно привести еще несколько примеров, доказывающих неоднородность группы *Heterotrichida*. Так, например, *Plagiotoma* проявляет ультраструктурные признаки, присущие представителям отр. *Hypotrichida* (Puytorac et al. 1976). *Sicuophora* (Puytorac et Grain 1968) имеет весьма своеобразное строение эктоплазматической фибриллярной системы, в связи с чем также появляются сомнения относительно принадлежности

этой инфузории к отр. *Heterotrichida*. По-видимому, необходима ревизия систематики гетеротрих, основанная на новых фактах, которые получены или будут получены с помощью электронной микроскопии.

SUMMARY

A study was made of the ciliary and fibrillar structures of *Bursaria truncatella*. The investigations show that by the cortex structure, the organization of the ectoplasmic and fibrillar systems, as well as by a number of structural peculiarities *B. truncatella* differs basically from the ciliates of the order *Heterotrichida* and on a whole from protozoans of the subclass *Spirotricha*.

By a number of morphological characters *Bursaria* should be included (in the bounds of the C o r l i s s system — C o r l i s s 1975) into the class *Kinetofragminophora*, subclass *Vestibulifera* and singled out as a special order *Bursaridida* related to the order *Colpodida*. Within our macrosystem (S e r a v i n and G e r a s s i m o v a 1977) *B. truncatella* is transferred from the subclass *Spirotricha* to the subclass *Homotricha*.

The expediency of revision of the *Heterotrichida* taxonomy is suggested.

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ПОДПИСИ К ТАБЛИЦАМ I—IX

Кортекс *Bursaria truncatella* O. F. Müll. по методу Шаттона и Львова в модификации Corliss (1957)

1–2: взаимное расположение кинетосомальных рядов на вентральной стороне тела у верхнего края перистомальной впадины справа (1) и слева (2) от края вентральной выемки, т.е. на левой и правой губах выемки. Общее увеличение 1500.
3: участок спинной поверхности. Общее увеличение 1500.
4: мембранеллы. Общее увеличение 1900.

Электронномикроскопическое строение кортекса *Bursaria truncatella*

5: поперечный срез кортекса в зоне реснички, × 25 000
6: косо-тангентальный срез кортекса, × 40 500
7–8: поперечный срез триады кинетосом, × 103 000 и × 108 000
9: поперечный срез кортекса в зоне реснички. × 102 000
10: продольный срез постцилиодесмы, × 53 000
11–13: поперечные срезы клетки в зоне адоральных мембранелл, × 6000, × 60 000 и × 16 000
14: поперечный срез мембранеллы, × 33 000
15–16: косые срезы через ретикулярную сеть, × 20 000 и × 64 000
17: поперечный срез мембранеллы, × — ось, на которой лежит триада кинетосом в мембранелле, X$_I$ — продольная ось мембранеллы, × 68 000
18: тангентальный срез в зоне септума, × 28 000
19: попреечный срез в зоне септума, × 40 000

Обозначения к таблицам I–IX: Ks — кинетосома, Kd — кинетодесмальный филамент, T — трансверсальная фибрилла, Pcd — постцилиодесма, Pc — постцилиарная фибрилла, Ep — эпиплазма, As — альвеолярный слой, R — пакет корневых нитей, Rr — ретикулярная сеть, Mb — мембранелла, U — ундулиподия, Um — унитарная мембрана, Mp — мукополисахаридные включения.

EXPLANATION OF PLATES I–IX

Cortex of *Bursaria truncatella* O. F. Müll., silver impregnated by the method of Chatton and Lwoff, modified by Corliss (1957)

1–2: Arrangement of kinetosomal rows on the ventral body side at the anterior border of peristomal convavity, from the right (1) and the left (2) side of ventral depression, i.e., on the right and the left lip. 1500 ×
3: phragment of the dorsal surface. 1500 ×
4: Membranelles. 1900 ×

Electron microscopical structure of *Bursaria truncatella* cortex

5: Transverse section through the cortex at the zone of a cilia. 25 000 ×
6: Oblique tangental section through the cortex. 40 500 ×
7–8: Transverse section through the triade of kinetosomes. 103 000 × and 108 000 ×
9: Transverse section through the cortex at the zone of a cilia. 102 000 ×
10: Longitudinal section through the postciliodesme. 53 000 ×
11–13: Transverse sections through the zone of adoral membranelles. 6000 ×, 60 000 ×, and 16 000 ×
14: Transverse section through the membranelle. 33 000 ×
15–16: Oblique sections through the reticular net. 20 000 × and 64 000 ×
17: Transverse section through the membranelle. x — axis to which the triade of membranelle kinetosomes is arranged, x_1 — longitudinal axis of the membranelle. 68 000 ×
18: Tangental section through the zone of septum. 28 000 ×
19: Transverse section through the zone of septum. 40 000 ×

Explanations: Ks — kinetosome, Kd — kinetodesmal filament, T — transversal fibrills, Pcd — postciliodesme, Pc — postciliary fibrills, Ep — epiplasma, As — alveolar layer, R — bundle of root filaments, Rr — reticular net, Mb membranelle, U — undulipodia, Um — unitary membrane, Mp — mucopolysaccharide inclusion.
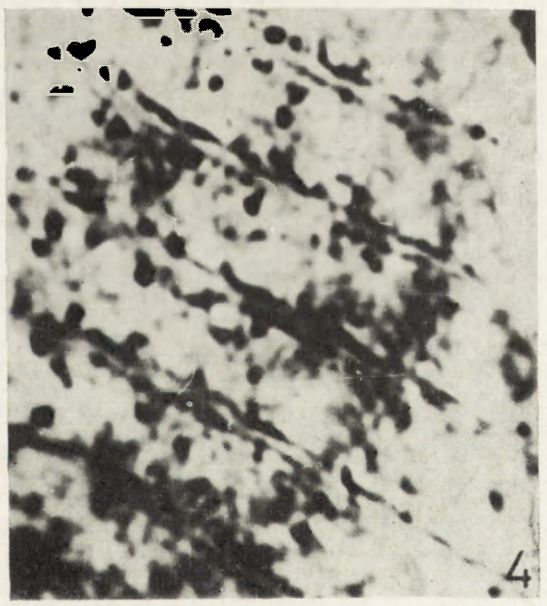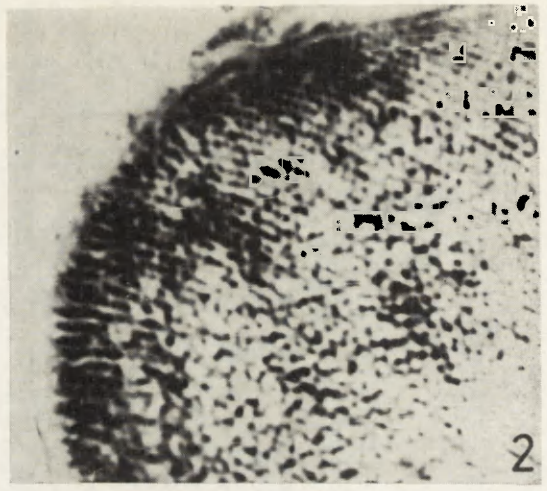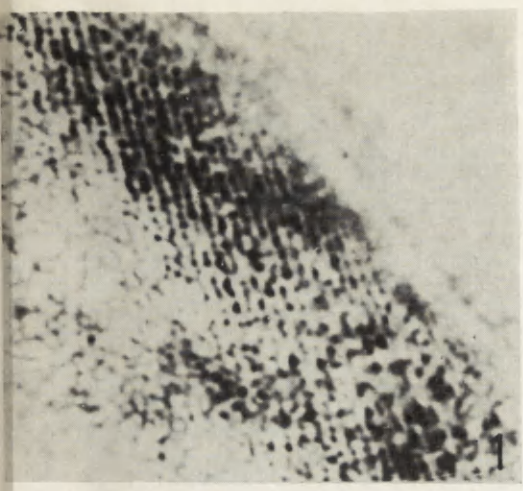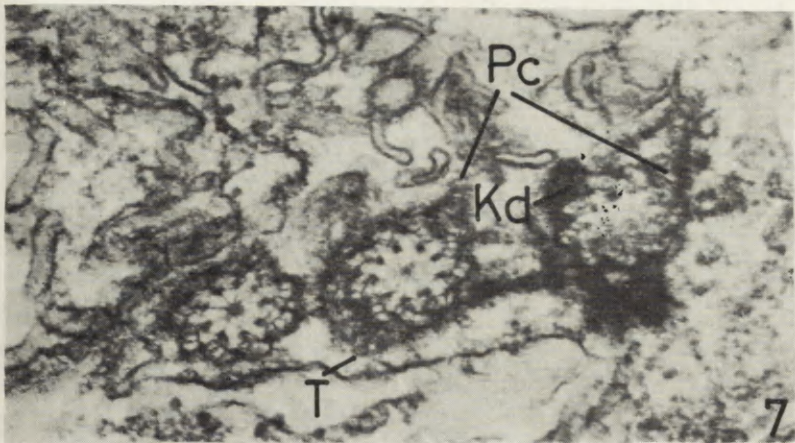
PLATE I

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PLATE II

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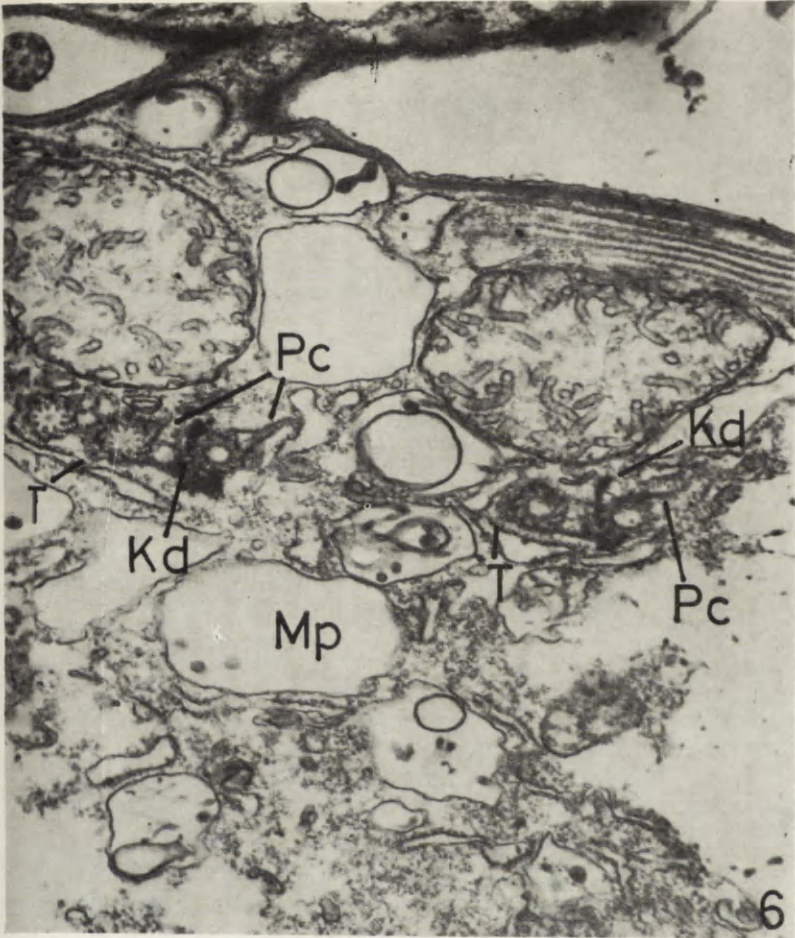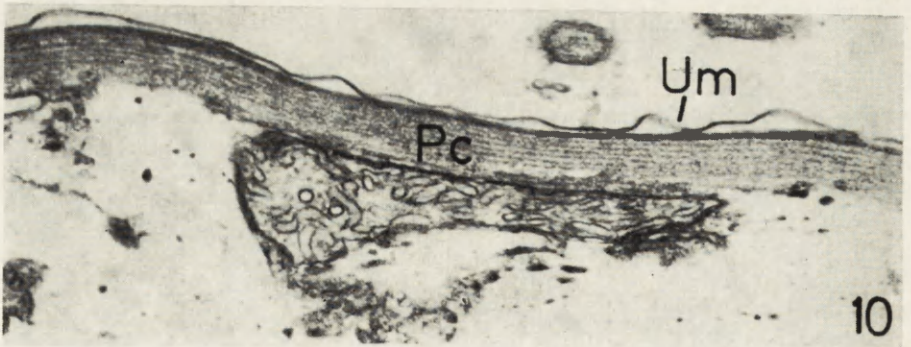
PLATE VI
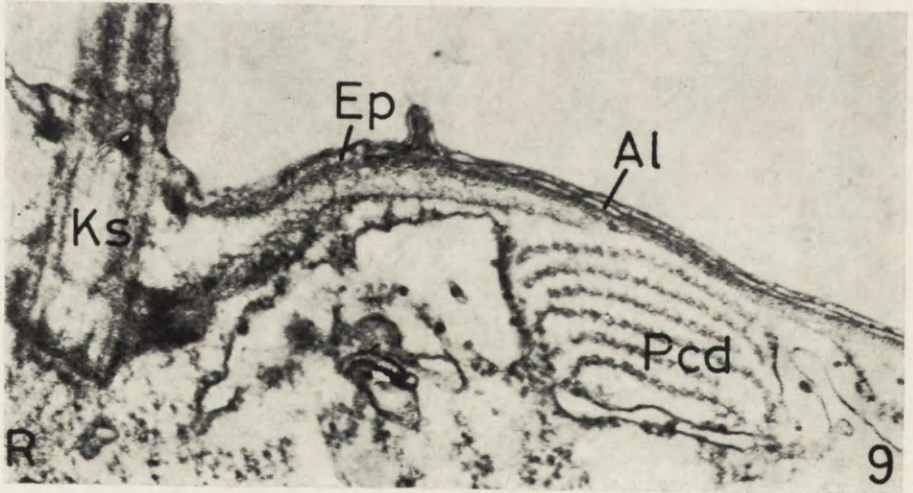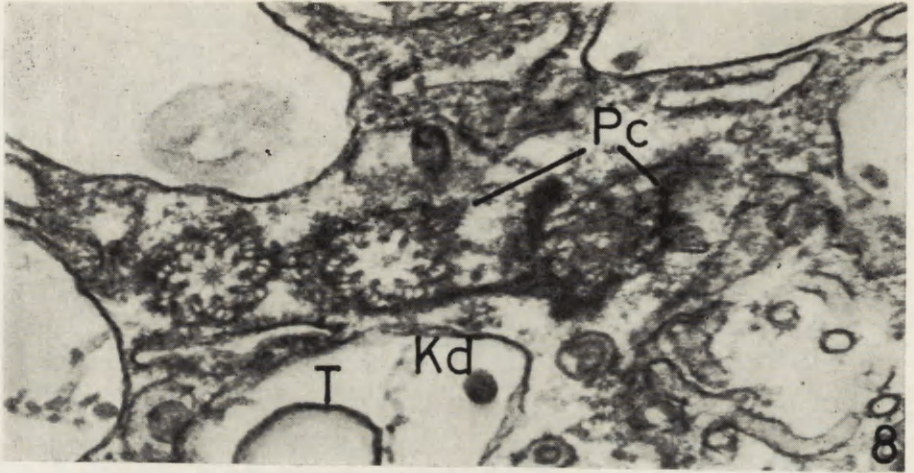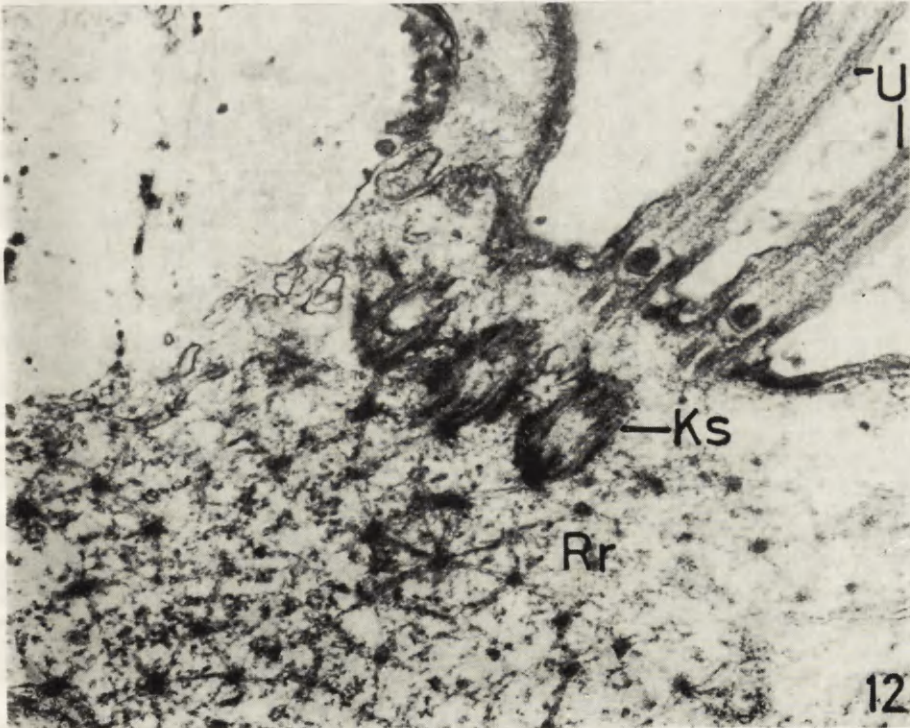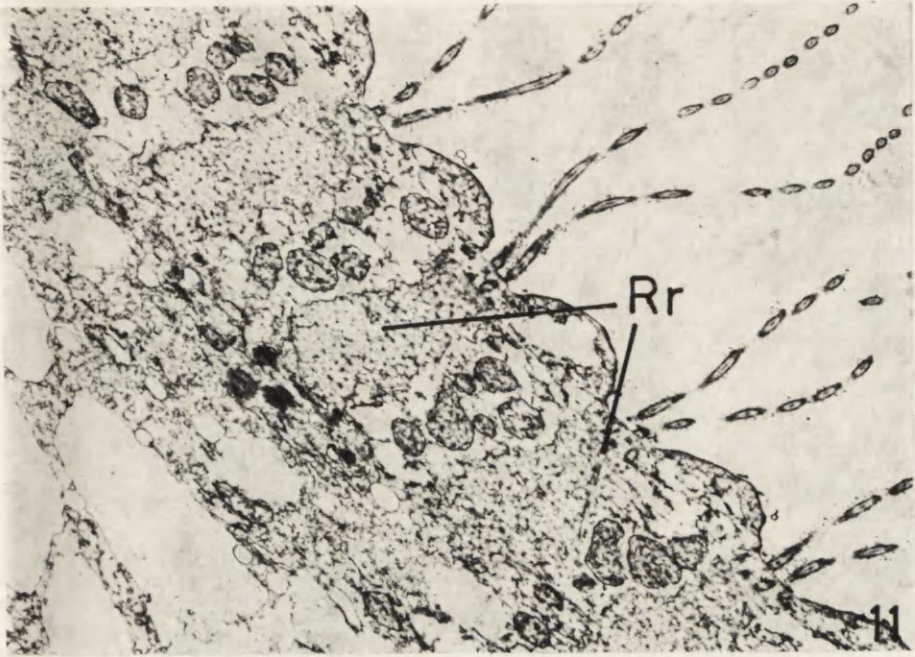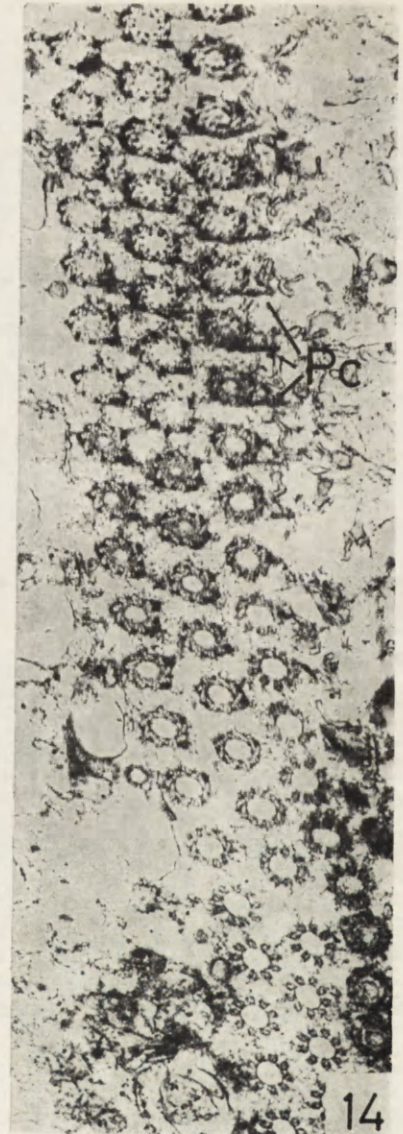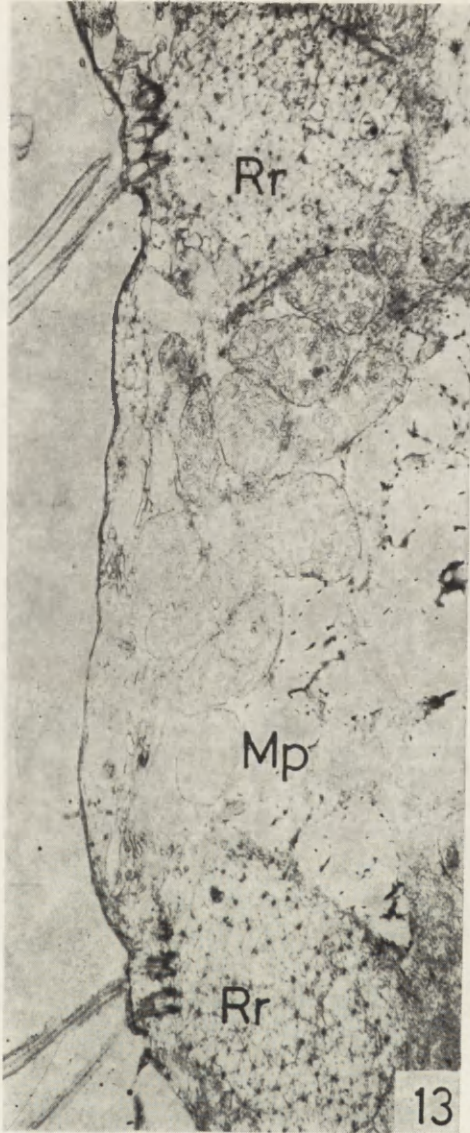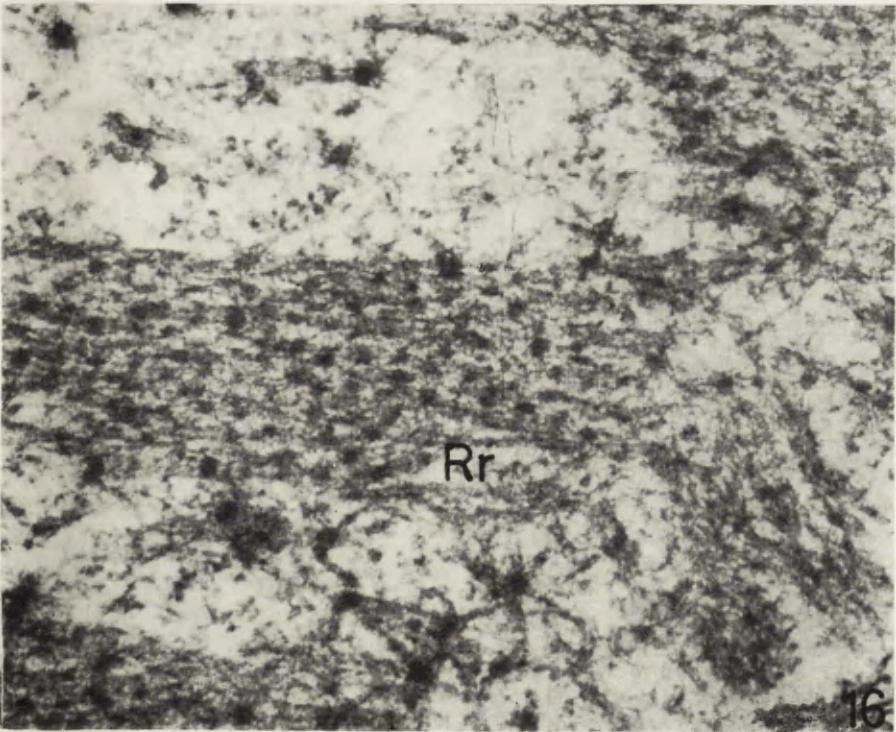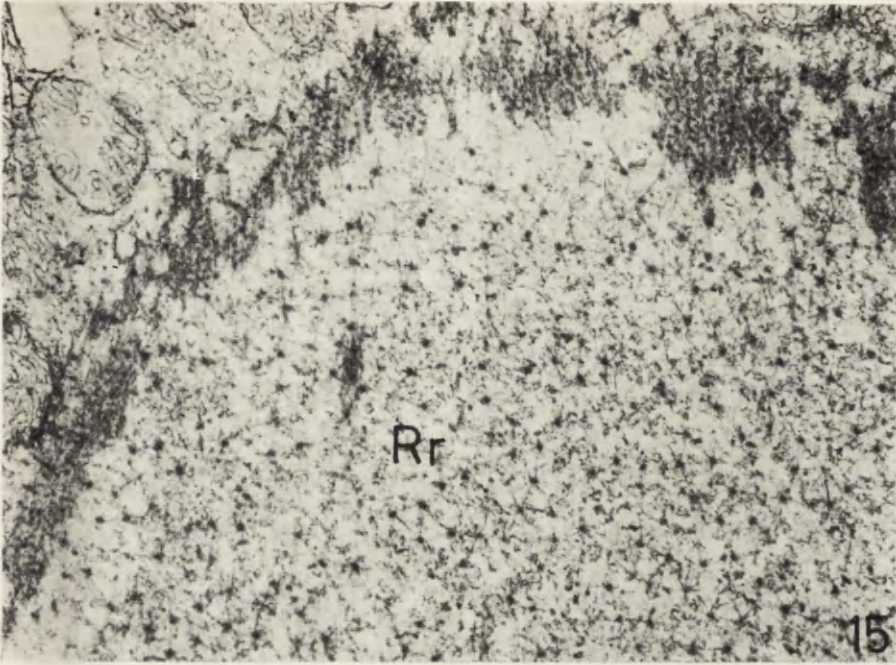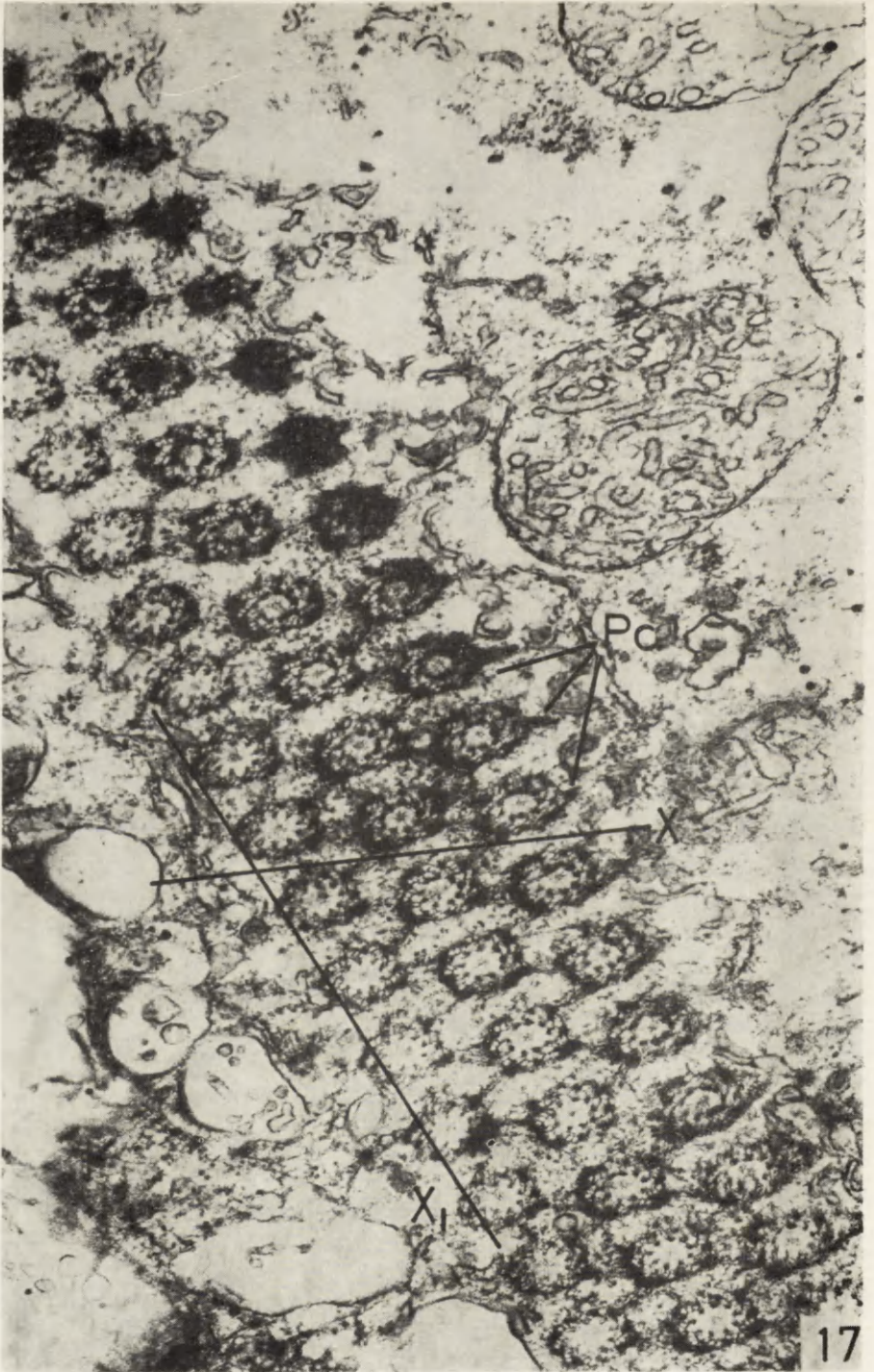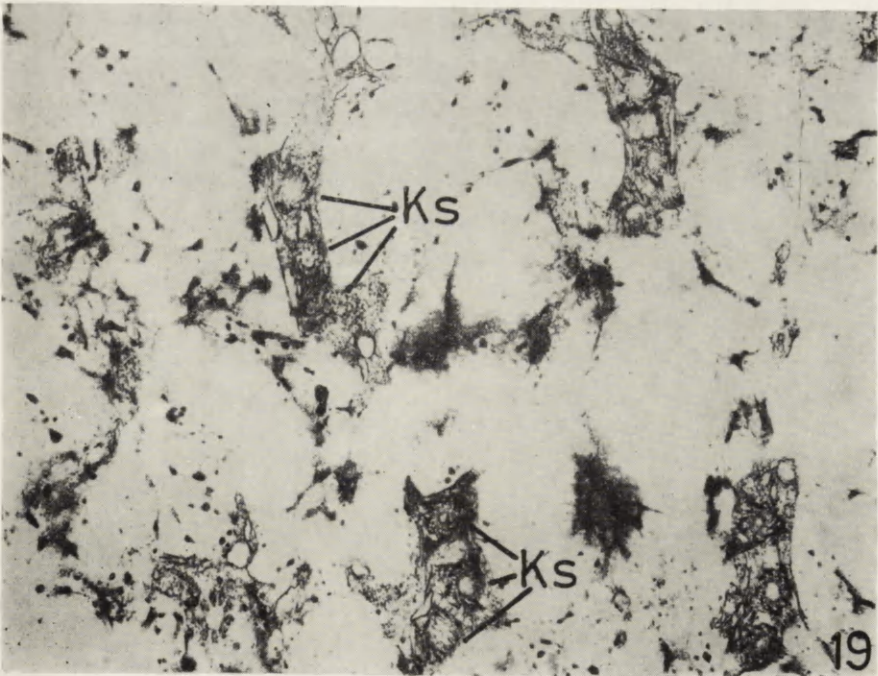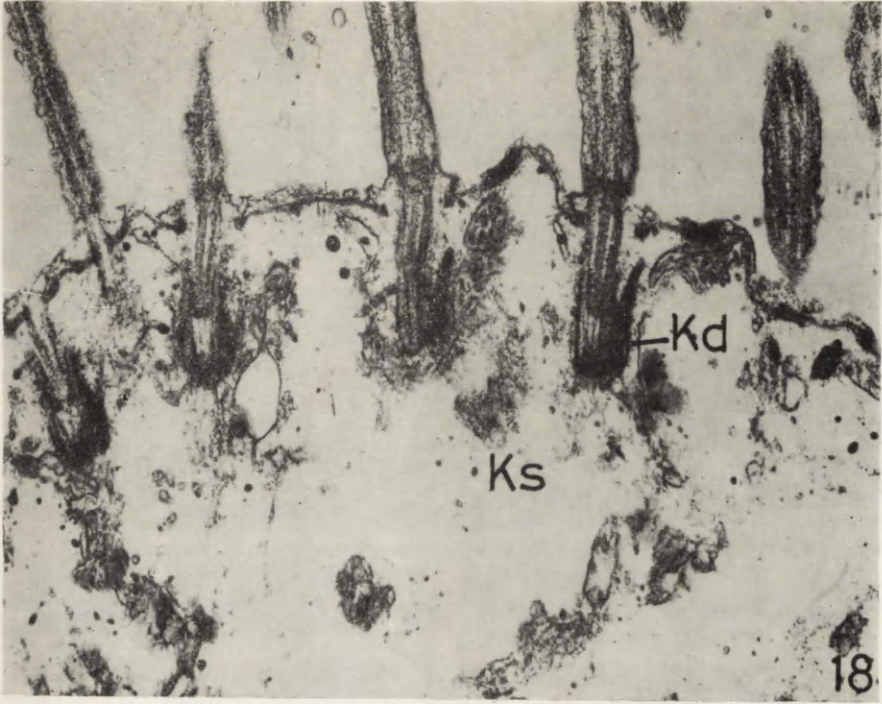

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Stanisław L. KAZUBSKI

Trichodina vesicularum Fauré-Fremiet, 1943 and T. *faurefremieti* nom. nov. (*Ciliata, Peritrichida*) — Parasites of Newts of the Genus *Triturus*

Synopsis. The trichodinas occurring in the urinary bladder of European newts, *Triturus vulgaris, T. montandoni, T. helveticus* and *T. cristatus* are described. Two species are distinguished within them: *Trichodina vesicularum* Fauré-Fremiet and T. *faurefremieti* nom nov. (syn. *T. urinicola*: F a u r é - F r e m i e t 1943). Morphological differences between these two species are discussed and diagnoses of species occurring in the urinary bladder of newts, including *T. bohemica* Haider are given. A critical review of the up to date findings of trichodinas from the urinary bladder of newts is also given as well as some remarks on host specificity of these ciliates.

There are only scarce informations on trichodinas parasitizing the urinary bladder of amphibians of the genus *Triturus*. First mention about these ciliates may be found in the paper by D a v a i n é (1854) and R o s s e t e r (1886). But F a u r é - F r e m i e t (1943) was the first who described in detail the trichodina species occurring in the urinary bladder of newts in France. One of these species, occurring in *T. cristatus* has been determined by this author as *Trichodina urinicola*, the name given by F u l t o n (1923) to the trichodinas parasitizing in toads *Bufo* sp. in USA. Another species, found in *T. vulgaris* and *T. palmatus* received a new name *T. vesicularum*. In the second extensive paper devoted to this group of ciliates L o m (1958) has stated that the trichodinas from newts belong to only one species, bearing the name *T. urinicola* due to the priority rule. However, he discerned three forms within this species, namely f. *bohemica* and f. *typica* both in *T. cristatus* and f. *taeniatus* in *T. taeniatus*. Some years later H a i - d e r (1964) raised these forms to the rank of subspecies.

The aim of the present work is to consider once more the problem of the distinctness and the systematic status of trichodinas occurring in the urinary bladder of European newts.

Table 1

A list of examined newts of the genus *Triturus*, harbouring trichodinas in the urinary bladder

Host	Locality	Date	No. of examined total	♂	♀	No. of infected total	♂	♀
T. vulgaris	environs of Warszawa – Łomna – Sulejówek	23.04.1958	30	24	6	–	–	–
		5.05.1958	3	2	1	2	1	1
	Olsztyn-Kortowo	6.05.1958	1	–	1	–	–	–
	Mazurian Lakeland – Święcajty lake	24.05.1958	24	6	18	–	–	–
	Olsztyn-Kortowo	30.05.1958	4	–	4	–	–	–
	environs of Warszawa – Łomna – Łuże	1 and 23.04.1959	2	–	2	–	–	–
		18 and 29.04.1959	2	–	2	–	–	–
	Grabie near Rawka river	30.04.1960	1	–	1	–	–	–
	Michałówka near Puławy	1.05.1951	1	–	1	–	–	–
	Pruszków	9.05.1962	1	1	–	–	–	–
	environs of Warszawa – Łuże	22.05.1954	9	7	2	6	6	–
	Olsztyn-Kortowo	24.07 and 3.08.1954	4	1	3	1	1	–
	Mazurian Lakeland – Ogonki near Węgorzewo	22.07.1968	2	1	1	1	1	–
		2.07.1970	2	1	1	1	1	–
	environs of Pruszków – Orębusy	17.03.1975	2	–	2	–	–	–
T. montandoni	Beskid Wyspowy – Luboń Wielki	20.05.1959	1	1	–	–	–	–
	Beskid Wysoki – environs of Nowy Targ	23.05.1959	27	11	16	12	8	4
	Pieniny	26.05.1959	5	4	1	3	3	–
	Pogórze Łupkowskie – environs of Sanok	28.05.1959	8	6	2	6	6	–
	Beskid Śląski – environs of Wisła	14–17.05.1960	10	5	5	4	3	1
	environs of Koniaków	18–20.05.1960	7	3	4	3	3	–
	Beskid Średni – Babia Góra							
	Zawoja-Markowa	3.06.1974	1	1	–	–	–	–
	Zawoja-Markowa	10.07.1974	1	–	1	1	–	1

Marków Stawek	24.07.1974	1	1	1	1
Zawoja-Markowa	4.08.1975	3	3	3	—
Zawoja-Markowa	16–20.08.1975	2	2	2	—
Marków Stawek	5.08.1975	9	8	7	1
T. helveticus Richelieu, dep. Indre et Loire, France	21–25.07.1960	4	2	1	1
	8.09.1960	8	—	—	—
environs of Besse-en-Chandesse, dep. Puy de Dôme, France	15.07.1976	8	3	2	5
	25.07.1976	8	3	2	2
T. cristatus Bieszczady – environs of Komańcza	16.06.1958	1	1	—	—
Pogórze Łupkowskie – environs of Sanok	28.05.1959	3	3	2	2
environs of Warszawa	23.05.1964	4	2	2	—

Moreover 55 individuals of *Triturus alpestris* were examined: 5 newts from Świętokrzyskie Mts. (14.05.1959); 10 from Luboń Wielki, Beskid Wyspowy Mts. (25.05.1959); 15 from the environs of Wisła (14–17.05.1960) and 17 from Koniaków (18–20.05.1960), Beskid Śląski Mts; 1 from Zawoja-Markowa (10.07.1974) and 3 from Marków Stawek (5.08.1975), Babia Góra Mt.; as well as 4 newts from France – environs of Besse-en-Chandesse, dep. Puy-de Dôme (3 newts or 15.07.1976 and 1 on 3.08.1976). No trichodinas were found in the urinary bladder of *T. alpestris* despite of the frequent occurrence of this newt species together with *T. montandoni* and *T. helveticus.*

Material and Methods

The trichodinas being the subject of the present investigation are the parasites of the urinary bladder of newts of the genus *Triturus* Raf. They have been collected from *T. cristatus* (Laur.), *T. vulgaris* (L.), and *T. montandoni* (Boulenger) from many localities in Poland as well as from *T. helveticus* (Razoumovsky) from France, from the environs of Richelieu (dep. Indre et Loire) and Besse-en-Chandesse (dep. Puy de Dôme). A detail list of localities and the data on infection of newts are given in Table 1.

The preparations were made according to Klein's silver impregnation method. From each subpopulation originating from one newt 30 ciliates have been examined. From some less numerous subpopulations, or when the preparations were not good enough, somewhat smaller number of ciliates was used. In each case the ciliates in course of division and the young ones have been omitted. A detailed statistical analysis of the material is given in the next paper on these trichodinas dealing with morphological variability (K a z u b s k i 1979).

A number of preparations stained with nuclear dyes (Meyer's haematoxylin and Feulgen reaction), both used after fixation in Schaudinn's fluid have been also used. For dimensions of the macronucleus samples of 10 ciliates from single host individuals have been measured. These samples were taken from various host species and localities within the distribution area. Before summarizing of these data it has been proved that there are no essential statistical differences between the means from particular samples.

Results

The morphometric data concerning cell size and the elements of the adhesive disc of trichodinas from *Triturus vulgaris*, *T. montandoni*, *T. helveticus* and *T. cristatus* are given in Table 2, while in Table 3 the dimensions of the macronucleus of ciliates from *T. vulgaris*, *T. montandoni* and *T. cristatus* are comprised. Unfortunately it was impossible to determine the situation and size of the micronucleus.

The morphology of examined trichodinas is represented in Figs. 1 and 2.

Discussion

The material given in Table 2 clearly shows that the trichodinas occurring in *T. vulgaris*, *T. montandoni* and *T. helveticus* have very similar body dimensions and the number of denticles. Also the structure of their adhesive disc and the shape of denticles (Fig. 1) show great similarity.
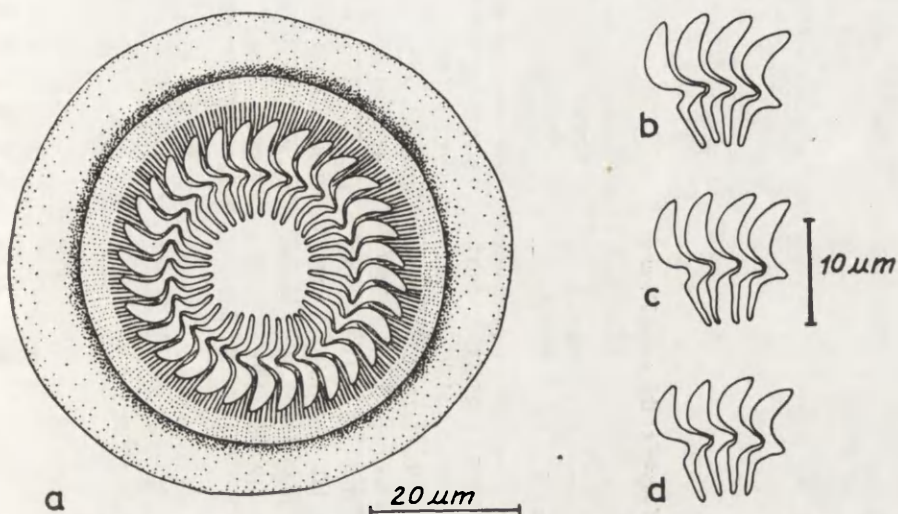
Fig. 1. *Trichodina vesicularum* Fauré-Fremiet, a — adhesive disc, b — denticles of ciliate from *Triturus vulgaris*, c — from *T. montandoni* and d — from *T. helveticus*
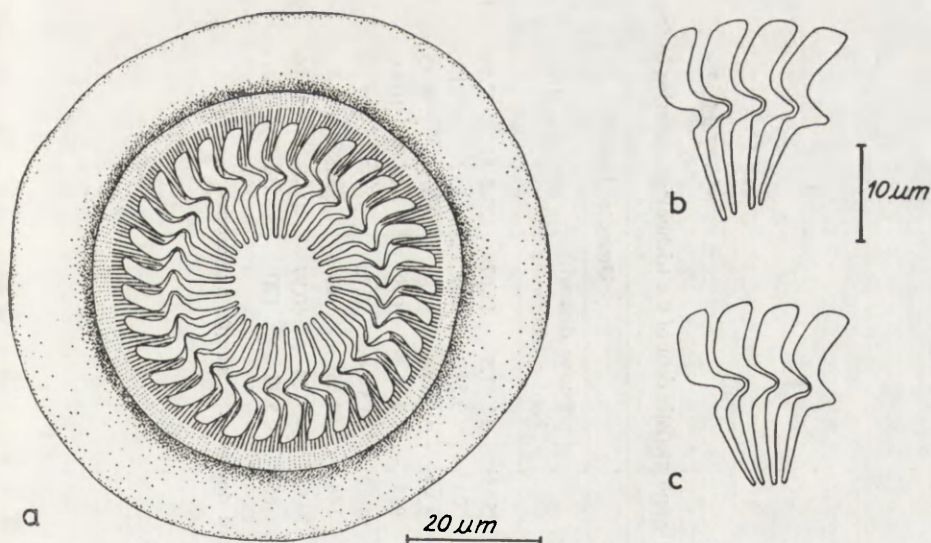
Fig. 2. *Trichodina faurefremieti* nom. nov., a — adhesive disc, b and c — denticles of ciliate from *Triturus cristatus*

The trichodinas from *T. cristatus* differ from these mentioned above. Being similar in regard of the body dimensions they have much more greater adhesive disc and somewhat greater number of denticles (Table 2). The denticles are also longer. There are also some differences in the general view of the adhesive disc (Fig. 2) and in the shape of

Table 2

Metric and meristic data of the trichodinas occurring in four species of newts of the genus *Triturus*

Host species	Diameter [μm]				No of denticles	Length of denticle [μm]
	body	adhesive disc with border membrane	adhesive disc	denticulate ring		
T. vulgaris	55–118[a] 75.19[b] / 9.64[c] 203[d]	34–51.5 45.67 / 3.54 209	29.5–51 37.99 / 4.12 218	18.5–33 22.35 / 1.62 222	25–35 28.77 / 1.96 222	9.5–17 11.77 / 1.38 180
T. montandoni	46–120 76.99 / 13.40 928	37.5–66 47.63 / 4.61 1079	30–55 38.32 / 4.01 1099	16–14 23.50 / 3.03 1104	24–35 29.86 / 2.03 1099	10–18 12.50 / 1.42 1041
T. helveticus	55.5–150 91.12 / 18.51 148	40.5–64 50.38 / 4.68 163	32–57 42.84 / 5.00 176	20.5–34 27.49 / 3.09 178	24–33 29.35 / 1.51 178	10–17 13.21 / 1.38 149
T. cristatus	59–103 79.95 / 9.82 85	49–74 60.97 / 5.42 120	44–61 51.80 / 4.11 120	24.5–40 34.62 / 2.96 119	27–36 31.99 / 1.97 120	14–21 17.48 / 1.86 120

[a] range, [b] mean, [c] standard deviation, [d] sample numerosity

the denticles. The denticles of the trichodinas from *T. vulgaris*, *T. montandoni* and *T. helveticus* are fairly slender, with the outer blade somewhat longer than the inner ray. Moreover, the blade gradually narrows toward the distal end, so the blade has the greatest width near its basal part. While in the trichodinas from *T. cristatus* both parts of the denticle — the blade and the ray, are equal or the ray is longer. Also the shape of the blade is different — it is almost of the same width at the whole length tapering at a small distance from the tip.

Similar differences concern also the dimensions of the nuclear apparatus (Table 3). The macronucleus of the trichodinas from *T. vul-*

Table 3

Dimensions of the macronucleus in trichodinas occurring in three species of newts of the genus
Triturus

Host species	Diameter of the macronucleus [μm]		Width of the macronucleus [μm]	
T. vulgaris	36–41[a]	36.80[b]	3–6[a]	4.82[b]
	2.33[c]	20[d]	0.67[c]	20[d]
T. montandoni	30–40	35.55	3–5	4.62
	2.80	20	0.46	20
T. cristatus	40–61	49.40	4–7	5.67
	5.54	40	0.62	40

[a] range, [b] mean, [c] standard deviation, [d] sample numerosity

garis and *T. montandoni* is very similar in dimensions, being clearly different from that of the trichodinas from *T. cristatus*. The latter is significantly larger than the macronuclei of trichodinas from the first two host species.

The above discussed data clearly show that we are dealing with two distinct species: the one occurring in *T. vulgaris*, *T. montandoni* and *T. helveticus*, and the other in *T. cristatus*. These species may be identified with *Trichodina vesicularum* and *T. urinicola* as described by F a u r é - F r e m i e t (1943). This is evident when we compare the body dimensions and drawings of the present material with the data given in the mentioned paper, especially with drawings of denticles (F a u r é - F r e m i e t 1943, Fig. 2 c, d).

This statement involves a problem of nomenclature. One of these two species, regarded by F a u r é - F r e m i e t as a new one, has been named *Trichodina vesicularum* Fauré-Fremiet, 1943, and this is a valid name. The other species according to F a u r é - F r e m i e t (1943) bears

Table 4
A critical review of the trichodinids occurring

Name used and reference	Diameter [μm]			No. of denticle	Length of denticle [μm]
	body	adhesive disc	denticulate ring		
Name lacking Davaine, 1854	—	—	—	29–30	—
Trichodina sp. Rosseter, 1886	63.5	—	—	30	—
Trichodina vesicularum Faure-Fremiet, 1943	50	—	19–22	26[a] 22–32[b]	—
Trichodina urinicola Fulton, 1923: Fauré-Fremiet, 1943	—	—	34–35	31 and 32 27–35	17.5
Trichodina urinicola. Fulton (?): Canella, 1954	—	—	—	32 and 33	—
Trichodina urinicola f. *bohemica*: Lom, 1958[d]	80 64–85	64 52–79	42 36–53	34 29–42	18.4
Trichodina urinicola f. *typica*: Lom, 1958[d]	80 64–85	52 45–60	32 29–35	31 28–36	18.4
Trichodina urinicola f. *taeniatus*: Lom, 1958[d]	75 60–80	42 36–44	22 21–24	30 27–34	14
Trichodina urinicola Raabe, 1959	—	42 30–55	25 13–30	29 26–31	
Trichodina urinicola sub sp. *urinicola*: Haider, 1964	59.6–84.1	42.8–67.3	27.5–35.1	27–36	
Trichodina urinicola sub. sp. *vesicularum*: Haider, 1964	61.2–81.0	38.25–47.4	21.4–26.01	24–33	
Trichodina vesicularum Fauér-Fremiet, 1943: Kazubski, present paper	78.58[a] 14.13[c]	38.68 4.37	23.73 3.19	29.64 2.02	12.47 1.38
Trichodina faurefremieti nom. nov., present paper	79.95 9.82	51.80 4.11	34.62 2.96	31.99 1.97	17.48 1.87

[a] mean or mode, [b] range, [c] standard deviation, [d] The same data have been repeated in the papers L o m 1959 and V o j t k o v a 1976

in the urinary blader of newts of the genus *Triturus*

Body hight [μm]	Macronucleus shape and dimensions	Host	Country	Proper name
—	—	*T. cristatus*	France	*T. faurefremieti*
50	—	*T. cristatus*	Great Britain	*T. faurefremieti*
—	horse-shoe-shaped	*T. vulgaris* and *T. palmatus* (= *T. helveticus*)	France	*T. vesicularum*
—	horse-shoe-shaped	*T. cristatus*	France	*T. faurefremieti*
—	—	*T. cristatus*	Italy	*T. faurefremieti*
65	horse-shoe-shaped with adjacent micronucleus	*T. cristatus*	Czechoslovakia	*T. bohemica*
65	horse-shoe-shaped, but sometimes U-shaped, with adjacent micronucleus	*T. cristatus*	Czechoslovakia	*T. faurefremieti*
60	horse-shoe-shaped with adjacent micronucleus	*T. taeniatus* (= *T. vulgaris*)	Czechoslovakia	*T. vesicularum*
—	—	*T. vulgaris*	Poland	*T. vesicularum*
62	U-shaped	*T. vulgaris* *T. cristatus*	GDR	*T. faurefremieti*
58.1	—	*T. vulgaris*	GDR	*T. vesicularum*
—	horse-shoe-shaped diameter 30-41	*T. vulagirs* *T. montandoni* *T. helveticus*	Poland France	*T. vesicularum*
—	horse-shoe-shaped diameter 40-61	*T. cristatus*	Poland	*T. faurefremieti*

the name *Trichodina urinicola* Fulton, 1923, used primarily for the trichodinas from the urinary bladder of *Bufo* sp. from Massachussetts, USA. Subsequently this name has been used by L o m (1958), R a a b e (1959), H a i d e r (1964) and others for trichodinas from newts. However, there are premises that the use of this name is not justified. *Trichodina urinicola* from *Bufo* sp. from the USA has a very high body — it has been marked by F u l t o n (1923) in the explanation to photograph (Fig. 9): "on account of the great body length, all the individuals of the preparations have the long axis parallel to the slide, so that the invaginated sucking disc is always seen inside view". According to photographs (F u l t o n 1923, Figs. 9 and 10) the body height of these trichodinas is about twice as great as the width while the trichodinas from the urinary bladder of *Triturus cristatus* according to F a u r é - F r e m i e t's and present author's observations, are much more lower — their body height does not overpass the width of their body. Another argument against the identity of both these trichodinas is their occurrence in different host species belonging to remote systematic groups (*Anura* and *Urodela*) and in different continents. Up to date observations show that the trichodinas especially those occurring in the urinary bladder of fish and amphibians, are strictly specific towards their hosts. In consequence, both species ought to be regarded as distinct ones. The trichodinas from *Bufo* sp. from the USA ought to retain the name *T. urinicola* Fulton, 1923 while for the trichodinas from the urinary bladder of *Triturus cristatus* from Europe, being the object of study by F a u r é - F r e m i e t and the present author, the new name *Trichodina faurefremieti* is proposed in honour of the great French protistologist. The names *T. urinicola* Fulton, 1923 sensu F a u - r é - F r e m i e t 1943, *T. urinicola* f. *typica* Lom, 1958 and *T. urinicola* subsp. *urinicola* Haider, 1964 fall into its synonyms.

There is one more problem concerning the trichodinas from *T. cristatus*. In this host species L o m (1958) has found another trichodina having greater dimensions of the adhesive disc and greater number of denticles. He has described it as a distinct form — *T. urinicola* f. *bohemica* (L o m 1958, Figs. 4, 6, ABC, 7) (subspecies *bohemica* according to H a i d e r 1964). These trichodinas, despite of the occurrence in the same host species and fairly great similarity to *T. faurefremieti* nom. nov., show some essential differences in the number and dimensions of denticles. It seems to be justified to recognize them as a distinct species which, according to the Code of Zoological Nomenclature, ought to bear the name *Trichodina bohemica* Haider, 1964.

Thus, in the urinary bladder of newts of the genus *Triturus* three species of trichodinas have been noted. The names, under which they

have been described, and the main morphological characters, as well as the distribution, references and their actual names are given in Table 4. The diagnoses of these species are as follows.

Trichodina vesicularum Fauré-Fremiet, 1943

(Synonyms: T. urinicola f. taeniatus: Lom, 1958; T. urinicola subsp. vesicularum: Haider, 1964)

The body of this trichodina is in shape of a flattened cone much more slant at one side, so that the projection of its tip on the plane falls at a distance from the base. Body dimensions and the number of denticles in ciliates collected from various hosts and localities in Europe are given in Tables 2-4. The structure of denticles is as follows. Both parts, outer blade and inner ray, are almost of the same length or the blade is slightly longer. The blade is slightly arch-shaped, gradually tapering towards the distal end. The widest part is in 2/3 of its length from the tip. The inner ray is fairly thin, sometimes slightly bent. The denticles including the blades, are proportionally narrow and the spaces between them are wide.

T. vesicularum occurs mainly in small newts: Triturus vulgaris (= T. taeniatus), T. montandoni, T. helveticus (= T. palmatus). The occurrence of this trichodina in T. cristatus is also probable.

Trichodina faurefremieti nom. nov.

(Synonyms: T. urinicola: Fauré-Fremiet, 1943; T. urinicola f. typica: Lom, 1958; T. urinicola subsp. urinicola: Haider, 1964)

The body of trichodina is in shape of a low, slant cone — the projection of its tip on the plane falls near the base. Body dimensions and the number of denticles of ciliates from various localities in Europe are given in Tables 2-4. The structure of the denticles is as follows. The outer blade and the inner ray are unequal in length. Usually the ray, fairly straight and solid, is slightly longer than the blade. The blade is fairly wide with both margins parallel at a proportionally long distance, sometimes even the blade becomes slightly wider in centrifugal direction. It tapers rapidly near the end so it looks sometimes as being truncated. The denticles are tightly arranged with fairly small spaces between neighbouring ones.

Host: Triturus cristatus.

Trichodina bohemica Haider, 1964

(Synonyms: *T. urinicola* f. *bohemica*: Lom, 1958; *T. urinicola* subsp. *bohemica*: Haider, 1964)

The body shape of this trichodina is from a regular hemisphere to bell or irregular conical. Drawings and photographs — cf. Lom (1958, Figs. 4, 6, ABC and 7). Body dimensions and the number of denticles are given in Table 4. The shape of denticles. The outer blade is slightly shorter than the inner ray. The blade tapers gradually and its tip is rounded. The ray is stright and fairly solid. Denticles are loosely arranged and the spaces between them are fairly large.

Host: *Triturus cristatus*. The species has been so far noted in Czechoslovakia (L o m 1958).

At the margin of the systematic and taxonomic problems concerning the trichodinas from newts some parasitological questions are worth to be mentioned. The considered species of trichodina show fairly narrow host specificity, especially *T. faurefremieti* and *T. bohemica* found exclusively in *Triturus cristatus*. *Trichodina vesicularum* is less specific being known to occur in *T. vulgaris*, *T. montandoni* and *T. helveticus*, sometimes even in *T. cristatus*. This species, however, has not been noted in *Triturus alpestris*, occurring in the same habitats as *T. montandoni*.

There is a mention pronounced by F a u r é - F r e m i e t (1943) that the trichodinas occur exclusively in male newts. My material ascertains this opinion to some extent (Table 1). The females of *T. helveticus* and *T. cristatus* were not infected but one out of 45 examined females of *T. vulgaris* harboured trichodinas in the urinary bladder. The infection rate in *T. montandoni* females was higher, attaining 25.8%. However in males the infection rate was as high as 79.6%. This phenomenon indicates the existence of some physiological or ecological mechanisms conditioning the infection of both sexes of newts by trichodinas.

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RÉSUMÉ

La description est donné des *Trichodina* trouvées dans les vessies des tritons: *Triturus vulgaris, T. montadoni, T. helveticus* et *T. cristatus.* On a constaté la présence de deux espèces: *Trichodina vesicularum* Fauré-Fremiet et *T. fauré-fremieti* nom. nov. (syn. *T. urinicola* Fauré-Fremiet, 1943). Les différences morphologiues entre ces deux espèces sont reexaminées. On donne les diagnoses spécifiques des *Trichodina* trouvées dans les vessies des tritons, *T. bohemica* Heider y comprise. Les rapports antérieurs concernant les *Trichodina* trouvées dans les vessies des tritons sont passés et révue et soumis à la discussion. On discute également la spécifité de ces parasites par rapport ù leurs hôtes.

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RÉSUMÉ

La description est donne des Trichodina trouvees dans les vessies des Triturus vulgaris, T. montandoni, T. helveticus et T. cristatus. On a constaté la présence de deux especes: Trichodina vesicularum Fauré-Fremiet et T. tabliaris nom. nov. syn. T. urinaria Fauré-Fremiet 1943). Les différences morpho-logiques entre ces deux especes sont réexaminées. On donne les diagnoses spéci-fiques des Trichodina trouvées dans les vessies des tritons, T. bohemica Haider y comprise. Les rapports numériques concernant les Trichodina trouvées dans les vessies des tritons sont passés et traite et compte à la discussion. On discute également la spécifité de ces parasites par rapport à leurs hôtes.

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Morphological Variability of *Trichodina vesicularum* Fauré-Fremiet and *T. faurefremieti* Kazubski, Parasites of Newts from Poland and France

Synopsis The source of morphological variability in ciliates *Trichodina vesicularum* Fauré-Fremiet and *T. faurefremieti* Kazubski, parasitizing in the urinary bladder of newts, have been approached by the analysis of variance. In the case of *T. vesicularum* fairly small geographical variability has been observed, concerning mainly the ciliates from the Central Massif (France), as well as the variability resulting from the altitude of habitats above sea level. Statistically significant variation has been found however only among particular subpopulations. This phenomenon seems to be connected with fairly high isolation of particular subpopulations of trichodinas inhabiting inner organs of their hosts. Similarly high variability among examined subpopulations has been noted in *T. faurefremieti*.

Trichodinas occurring in the urinary bladder of amphibians are a good subject for study of morphological variability. As a rule, these ciliates occur abundantly giving the possibility to examine many specimens from one host individual and to have a representative sample from each subpopulation. The comparison of such subpopulations being to a great degree isolated from each other due to their occurrence in internal organs, but having a possibility of contacts due to the contacts between their hosts, allows to recognize the intraspecific variability conditioned by various factors. Until now the trichodinas from the urinary bladder of newts of the genus *Triturus* have not been studied in this respect. The present paper is a complement to the previous study (Kazubski 1979) on the systematic position of two species of *Trichodina* from newts.

Table 1

Mean values (M) and standard deviations (SD) of main characters in samples of 47

Host	Locality	Altitude above sea level [m]	Date	No. of subpopulation	body		
					M	SD	*n*
Triturus vulgaris	Olsztyn — Kortowo Mazurian Lakeland —	80	24. 07. 1964	216	75.23	8.87	30
	Ogonki lake environs of Warszawa —	100	2. 07. 1970	230	72.03	8.42	30
	Łuże	100	22. 05. 1964	204	78.45	7.82	29
				211	74.06	10.23	27
	environs of Warszawa —	120	5. 05. 1958	32	75.64	11.49	28
	Sulejówek			33	73.79	11.33	17
Triturus montandoni	Pogórze Łupkowskie — environs of Sanok	350	28. 05. 1959	128	77.63	9.49	30
				130	74.82	6.32	30
				131	80.05	7.83	30
				132	86.80	12.97	30
				133	81.20	9.17	30
	Beskid Wysoki — environs of Nowy Targ	600	23. 05. 1959	103	101.92	7.81	30
				104	79.77	9.02	24
				105	79.98	8.70	30
				106	76.39	12.46	28
				107	78.47	8.90	29
				118	86.25	10.92	30
				120	86.60	10.63	21
	Pieniny	770	26. 05. 1959	126	74.04	8.48	24
	Beskid Śląski — environs of Koniaków	650–700	18. 05. 1960	178	86.66	11.48	29
				179	83.92	12.26	30
				193	73.83	7.78	30
	Beskid Śląski — environs of Wisła	600–700	14–17. 05. 1960	166	87.44	10.35	17
				168	87.75	11.12	22
				172	78.79	12.36	27
				156	86.96	10.31	27
	Beskid Średni — Babia Góra, Zawoja-Markowa	600	10. 07. 1974	240	80.36	8.56	29
			4. 08. 1975	242	82.23	11.50	30
				243	74.23	15.27	30
				244	82.77	16.18	30
			16. 07. 1975	258	64.83	8.15	30
			20. 08. 1975	259	61.83	8.11	24
	Beskid Średni — Babia Góra, Marków Stawek	1135	24. 07. 1974	241	81.42	7.98	30
			6. 08. 1975	245	58.79	7.56	14
				246	83.30	16.22	30
				247	76.45	9.22	19
				248	73.63	11.19	19
				249	71.68	14.54	30

subpopulations of *Trichodina vesicularum* from Poland and France (*n* — sample numerosity)

Diameter [μm]									No. of denticles			Length of denticle [μm]			Width of denticle [μm]
adhesive disc with border membrane			adhesive disc			denticulate ring									
M	SD	n	M	SD	n	M	SD	n	M	SD	n	M	SD	n	
45.13	3.37	30	34.68	2.16	30	21.37	1.35	30	28.17	1.51	30	12.08	0.82	30	2.38
43.92	2.84	30	34.00	1.90	30	21.17	1.24	30	28.20	0.92	30	11.85	0.60	30	2.36
43.74	2.97	27	38.93	2.92	27	22.12	1.19	30	28.83	0.87	30	11.05	0.52	11	2.41
45.22	3.39	30	35.13	2.68	30	22.08	1.65	30	27.47	0.94	30	11.80	0.78	30	2,53
47.84	2.19	25	43.55	2,43	29	23.33	0.96	30	29.57	1.04	30	11.58	0.51	19	2.48
47.91	4.21	28	39.00	3.79	28	23.66	0.86	28	29.68	0.86	28	12.04	0.73	26	2.50
49.10	3.93	30	37.45	4.31	30	23.02	3.21	30	29.03	1.97	30	12.15	1.35	30	2.49
45.63	1.97	30	35.02	1.69	30	21.93	1.28	30	29.20	0.92	30	11.63	0.60	30	2.36
46.93	2.04	30	35.65	2.28	30	21.68	1.37	30	27.80	1.00	30	12.08	0.76	30	2.45
52.02	3.77	30	41.85	3.91	30	26.75	3.03	30	30.00	1.23	30	13.98	1.52	30	2.80
48.38	3.25	30	38.37	4.37	30	24.18	3.30	30	28.53	2.28	30	12.87	1.38	30	2.66
58.12	2.72	30	47.00	3.13	30	30.40	2.45	30	32.67	1.65	30	15.02	1.24	30	2.92
47.03	3.06	30	37.70	2.88	30	24.22	2.40	30	28.73	1.86	30	12.80	1.34	30	2.65
49.12	4.50	30	36.95	4.11	30	23.53	2.83	30	29.80	2.51	30	12.93	1.12	30	2.48
43.36	3.03	25	37.23	2.87	30	22.20	1.40	30	30.10	1.30	30	11.10	0.88	10	2.32
46.59	3.35	29	35.45	3.19	30	22.17	2.50	30	29.73	1.20	30	12.12	0.92	30	2.34
49.70	3.99	30	37.68	4.16	30	24.13	2.91	30	30.17	2.02	30	12.50	1.36	30	2.51
44.09	2.61	28	35.33	1.92	30	22.42	1.93	30	29.80	1.21	30	11.90	0.77	30	2.36
50.43	4.54	30	39.17	3.93	30	25.00	4.71	30	30.67	1.45	30	12.92	1.33	30	2.56
52.67	3.87	30	41.33	4.25	30	26.60	3.44	30	30.60	1.69	30	13.58	1.42	30	2.73
53.28	4.34	30	43.85	3.56	30	26.70	2.52	30	30.33	1.65	30	14.95	1.95	30	2.77
48.00	3.32	30	36.22	2.74	30	23.15	1.84	30	29.17	0.95	30	12.02	0.79	30	2.49
48.52	4.69	27	39.20	3.10	30	25.22	1.84	30	31.60	1.57	30	12.28	0.78	30	2.51
50.02	3.97	30	39.62	4.16	30	25.27	3.24	30	30.50	1.50	30	12.85	1.03	30	2.60
46.13	3.28	30	35.72	3.06	30	22.90	2.40	30	30.07	1.46	30	11.72	1.12	30	2.39
50.17	3.45	30	40.67	3.52	30	25.42	2.16	30	30.67	0.92	30	13.17	1.25	30	2.60
45.58	3.14	30	39.98	3.29	30	21.45	1.90	30	27.87	0.90	30	12.75	0.72	30	2.42
46.85	3.18	30	39.87	2.89	30	22.72	2.00	30	30.77	1.48	30	13.30	0.89	30	2.32
44.62	2.62	30	37.60	2.64	30	21.48	2.05	30	29.63	1.43	30	12.25	0.81	30	2.28
46.15	2.48	30	39.02	2.41	30	22.85	1.52	30	30.73	1.51	30	12.60	0.79	30	2.34
47.72	2.27	30	35.73	1.92	30	23.33	1.52	30	30.40	1.16	30	11.07	0.55	30	2.41
46.73	3.09	30	36.13	3.01	30	22.43	2.10	30	28.13	2.05	30	11.42	0.87	30	2.50
43.83	2.80	30	37.53	2.62	30	20.92	1.19	30	30.28	1.00	29	11.50	0.91	24	2.17
44.48	2.59	30	37.00	2.51	30	22.17	1.64	30	29.87	1.41	30	11.93	0.96	30	2.33
49.28	3.47	30	42.33	2.74	30	26.28	2.60	30	31.33	2.35	30	13.52	1.35	30	2.64
46,72	2.64	30	39.30	2.43	30	23.90	2.10	30	30.43	1.50	30	12.93	1.21	30	2.47
47.50	2.14	25	37.82	2.24	25	23.04	2.12	25	29.32	1.55	25	12.40	0.99	25	2.47
44.58	2.41	30	36.28	2.03	30	22.47	1.43	30	30.07	1.23	30	11.75	0.61	30	2.35

Table 1 — concluded

				251	79.48	10.15	23
				256	66.71	11.09	12
				257	67.25	11.48	14
Triturus helveticus	Richelieu dep. Indre et Loire, France	150	21. 07. 1960	1	74.35	7.73	30
	environs of Besse-en-Chandesse dep. Puy de Dôme, France	1200	15. 07. 1976	129	89.03	11.61	20
				130	98.02	13.14	24
				131	109.96	16.10	24
			25. 07. 1976	137	76.57	14.36	27
				138	105.68	11.04	19
Poland — summarized data					77.91	8.12	41
France — summarized data					92.27	14.86	6
t_0					3.50		
					1%		
Summarized data					79.75	10.23	47

Material and Methods

The study has been made on ciliates *Trichodina vesicularum* Faure-Fremiet from *Triturus vulgaris*, *T. montandoni* and *T. helveticus* and on *Trichodina faurefremieti* Kazubski from *T. cristatus*. The source of particular subpopulations (the term used for ciliates originating from a single host specimen) is given in Table 1. The preparations were made according to Klein's dry method. From each subpopulation an arbitrarily determined sample of 30 specimens was examined, but some samples comprising 25-29 ciliates have been also used. It was not possible to observe all considered characters in some specimens of ciliates, so the numbers of measured individuals may differ in particular cases.

The following characters have been examined: (1) body diameter (DB), (2) diameter of the adhesive disc with border membrane (DDM), (3) diameter of the adhesive disc without border membrane (DAD), (4) diameter of the denticulate ring (DDR), (5) number of denticles (ND), (6) length of denticles (LD). Moreover, for each subpopulation the mean length of an arch of the denticulate ring circumference falling on a single denticle has been counted according to the formula:

$$\frac{\text{mean diameter of the denticulate ring}}{\text{mean number of the denticles}} \times \pi.$$

Then, the variablity was approached by statistical methods, mainly by the analysis of variance using "Two and multilevel nested ANOVA with unequal sample sizes" (S o k a l and R o h l f 1969, Box 10.4, 10.5). This analysis was performed for three characters regarded as the most representative ones: (1) diameter of the adhesive disc without border membrane, (2) diameter of the denticulate ring and (3) the number of denticles. In trichodinas all metric data are highly correlated so the examination of greater number of features would give no more effect. Also in the present investigation a significant convergence of results

45.62	2.92	29	39.64	3.17	29	22.05	1.68	29	29.59	1.59	29	11.63	0.93	26	2.34
44.57	2.34	30	35.87	2.48	30	22.03	1.80	30	30.00	1.05	30	11.65	0.96	30	2.31
45.20	2.88	30	35.82	2.56	30	21.80	2.11	30	28.30	1.44	30	11.78	0.99	30	2.42
45.40	2.15	30	36.07	1.57	30	23.03	1.04	30	28.00	1.36	30	12.03	0.61	30	2.58
51.35	3.24	30	42.68	2.44	30	28.15	1.85	30	29.20	1.42	30	13.53	1.26	30	3.03
54.11	2.91	27	45.87	2.36	27	30.00	1.79	27	30.26	1.38	27	14.30	1.38	27	3.11
56.05	3.64	18	49.33	3.15	30	30.38	2.29	30	30.03	1.19	30	12.56	0.73	8	3.18
48.17	3.93	30	39.67	3.22	30	26.35	2.57	30	29.33	1.30	30	12.95	1.18	30	2.83
50.32	3.44	22	44.26	2.40	23	27.72	1.17	25	29.44	1.45	25	14.00	1.61	18	2.96
47.38	3.03	41	38.21	2.79	41	23.40	1.95	41	29.70	1.10	41	12.38	0.92	41	2.47
50.90	3.87	6	42.98	4.67	6	27.61	2.69	6	29.36	0.79	6	13.23	0.87	6	2.95
2.57			3.57			4.71			0.73			2.13			6.65
5%			1%			1%			ns			5%			1%
47.83	3.32	47	38.82	3.43	47	23.94	2.47	47	29.66	1.07	47	12.49	0.95	47	2.53

obtained after the analysis of variance of two metric features — the diameter of the adhesive disc and of the denticulate ring, may be noticed. Such selection of characters allows to expect that the results obtained due to the analysis of variance would show highly significant differences.

In a series of cases the correlation between particular characters has been proved. The correlation was counted for the means from particular samples with the aid of TI-SR-51-II calculator.

The influence of geographical distribution, host species, altitude above sea level and, in the case of abundant material from Poland, the variability between local groups of subpopulations have been tested. The seasonal variability has not been tested because of the lack of longer series of ciliates from the same localities.

Results

Trichodina vesicularum Fauré-Fremiet, 1943 (Pl. I 1–10, II 11–14)

Mean values of the examined characters originating from 47 examined subpopulations are given in Table 1. For the first look there are great differences between the means of particular subpopulations with simultaneously small standard deviations. The only exception is the body diameter which shows fairly great standard deviation. This results from two reasons: (1) cell dimensions of trichodinas are more enlarging during the growth than the dimensions of the adhesive disc; (2) during dessication of the preparation soft parts of the cell are subjected to greater deformation than the skeletal elements of these protozoans.

Correlations and parameters of regression lines between mean values

Table 2

Correlation coefficient and regression line parameters between mean values of characters in examined
T. vesicularum subpopulations

Character	Correlation coefficient	Regression coefficient	Intercept
DB : DDR	0.754253	3.1291	4.8366
DDM : DDR	0,922017	1.2414	18.1061
DAD : DDR	0,865214	1.2020	10.0450
ND : DDR	0,494769	0.2137	24.5440
LD : DDR	0.778837	0.3002	5.2984
LD : ND	0.426090	0.3803	1.2050

For abbreviations see p. 388

of particular characters in subpopulations are given in Table 2. All metric characters, as the body diameter, adhesive disc diameter together and without border membrane, denticulate ring diameter and the length of denticles appeared to be highly correlated. Lower correlation, however, essentially different from 0, occurs between the number of denticles and the diameter of the denticulate ring as well as between the length and the number of denticles.

Dimensions of the adhesive disc, denticulate ring diameter. and the number of denticles in regard to host species and altitude of habitats above sea level are presented in Table 3. The vacant places in this table are due to the character of distribution of newts, the hosts of *Trichodina. Triturus vulgaris* is common in northern part of the Central Europe. In Poland it occurs in lowlands. *T. montandoni* is a Carpathian endemite; in highlands it may come in contact with *T. vulgaris. T. helveticus* occurs in West Europe, in lowlands and mountains as well. Thus, when the action of both agents, host species and altitude of habitats above sea level, is taken into account, the analysis of variance becomes very complicated and may be approached step by step, considering particular conditions in a hierarchic system.

The collected materials, despite of their fragmentary, have allowed to draw some more general regularities characterizing the species *Trichodina vesicularum*.

First of all fairly great differences ought to be noted between the trichodinas from Poland and France (Table 1). Having similar number of denticles the trichodinas from France are much larger than those from Poland. These differences are statistically important at the level of 5 or 1% (Table 1). The differences in size concern mainly the material from Central Massif (Tables 1 and 3). However, the analysis of

Table 3

Adhesive disc diameter (DAD), denticulate ring diameter (DDR) and number of denticles (ND) in *Trichodina vesicularum* depending on host species and altitude of habitat above sea level

		T. vulgaris		*T. montandoni*		*T. helveticus*		Summarized data	
lowlands	DAD	37.47[a]	4.285[b] 174 (6)[c]			36.07	1.574 30 (1)	37.27	4.032 204 (7)
	DDR	22.27	1.638 178 (6)			23.03	1.041 30 (1)	22.38	1.587 208 (7)
	ND	28.64	1.304 178 (6)			28.00	1.365 30 (1)	28.55	1.329 208 (7)
350 m	DAD			37.67	4.216 150 (5)			37.67	4.216 150 (5)
	DDR			23.51	3.168 150 (5)			23.51	3.168 150 (5)
	ND			28.91	1.722 150 (5)			28.91	1.722 150 (5)
600–800 m	DAD			38.64	4.286 630 (21)			38.64	4.286 630 (21)
	DDR			23.98	3.200 630 (21)			23.98	3.200 630 (21)
	ND			30.10	1.848 630 (21)			30.10	1.848 630 (21)
above 1100 m	DAD			37.95	3.238 264 (9)	44.34	4.310 140 (5)	40.16	4.744 404 (14)
	DDR			22.74	2.358 264 (9)	28.52	2.496 142 (5)	24.76	3.660 406 (14)
	ND			29.92	1.678 263 (9)	29.63	1.397 142 (5)	29.82	1.591 405 (14)
summarized data	DAD	37.47	4.285 174 (6)	38.32	4.052 1044 (35)	42.88	5.069 170 (6)	38.77	4.494 1388 (47)
	DDR	22.27	1.638 178 (6)	23.60	3.048 1044 (35)	27.56	3.111 172 (6)	23.92	3.247 1394 (47)
	ND	28.64	1.304 178 (6)	29.88	1.8333 1043 (35)	29.34	1.519 172 (6)	29.66	1.788 1393 (47)

[a] mean, [b] standard deviation, [c] number of specimens; in parenthesis, the number of subpopulations examined

variance of all three characters in trichodinas from lowlands of France and Poland has shown lack of difference between compared groups of ciliates $(F_0 < 1)$, except those existing among particular subpopulations. The differences between trichodinas originating from mountain habitats (above 1100 m) from Poland and France show highly significant differences concerning the diameter of the adhesive disc $(F_0 = 16.918)$ and the diameter of the denticulate ring $(F_0 = 40.645)$, but lack of difference

in the number of denticles ($F_0 < 1$). Simultaneously a high degree of differentiation occurs among particular subpopulations.

The correlation between mean numbers of denticles and mean dimensions of the denticulate ring in subpopulations of trichodinas from France and Poland has been also examined (Fig. 1). Both these groups
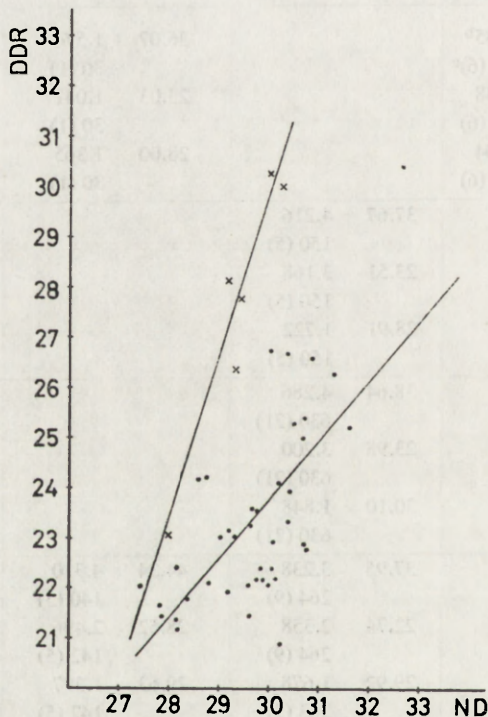
Fig. 1. Diagram of correlation and regression between mean diameter of denticulate ring (DDR) and mean number of denticles (ND) in subpopulations of *Trichodina vesicularum* from Poland (.) and France (×)

differ by the correlation coefficient, being significantly different from 0 in both groups, and by the parameters of the regression lines (Table 4). However, the comparison of regression coefficient (slope) by the parallelity test has shown that the differences between regression lines are significant but only at 10% level ($t_0 = 1.72417$).

Besides the differences of quantitative character between the trichodinas from Poland and France (mainly from the Central Massif) there are some differences in the dimensions and shape of denticles. In the trichodinas from the Central Massif the denticles are wider (Table 1) and have more solid centripetal part (Pl. II 12–14).

As the differences between *T. vesicularum* from Poland and from

Table 4

Correlation coefficients and regression line parameters between number of denticles and denticulate ring diameter in *T. vesicularum* subpopulations form Poland and France

	Poland $n = 41$	France $n = 6$
Correlation coefficient	0.666156	0.964774
Regression coefficient	1.177400	3.269114
Intercept	−11.5701	−68.3762

France have obviously geographical character it is justified to consider them separately.

In the trichodinas from Poland there are differences in the adhesive disc diameter, denticulate ring diameter and the number of denticles depending on the origin of ciliates from *Triturus vulgaris* and *T. montandoni* (Table 3). These differences are not so great and the ciliates may be aligned in a row common for both host species. This row shows an increase of values of examined characters, being parallel to growing altitudes above sea level. A breaking of this correlation is observed in the material originating from the highest habitats — there a slight decrease of the adhesive disc diameter and of the denticulate ring diameter may be observed as well as diminishing of the number of denticles. The analysis of variance in hierarchic system has comprised: (1) host dependent variability (mean values for particular host species are in Table 3) (2) variability dependent on the altitude of habitat above sea level (Table 3), (3) variability within local populations (means for local groups are in Table 5), and (4) variability between subpopulations originating from single host individuals (mean values for particular subpopulations are in Table 1). The analysis has shown (Table 6) that the first three conditions produce the variability not significant statistically, while the variability between particular subpopulations is highly significant. It ascertains previous mention about great significance of variabilty between particular subpopulations in this species of trichodina.

In the trichodinas from France, collected from *Triturus helveticus,* also the increase of the adhesive disc diameter, denticulate ring diameter and greater number of denticles together with growing altitude may be discerned (Tables 1 and 4). However, the analysis of variance has shown that the differences in the adhesive disc diameters are statistically insignificant ($F_0 = 4.3626$ at critical value $F_{0.05} = 7.71$) while the differences between the diameter of the denticulate ring and the number of denticles against the altitude of habitats are significant at

Table 5

Mean values (M) and standard deviations (SD) of three examined characters in local groups in *Trichodina vesicularum*

Locality	Diameter [μm]						No. of denticles		
	adhesive disc			denticulate ring					
	M	SD	n	M	SD	n	M	SD	n
Mazurian lakeland	34.34	2.05	60	21.27	1.29	60	28.18	1.24	60
environs of Warszawa	39.13	4.24	114	22.78	1.56	118	28.87	1.28	118
environs of Sanok	37.67	4.22	150	23.51	3.17	150	28.91	1.72	150
environs of Nowy Targ	38.19	4.91	210	24.06	4.15	210	30.14	2.05	210
Pieniny Mts.	39.17	3.93	30	25.00	4.70	30	30.67	1.45	30
Koniaków	40.46	4.76	90	25.47	3.12	90	30.03	1.58	90
Wisła	38.80	3.92	120	24.70	2.65	120	30.71	1.48	120
Zawoja	38.05	3.18	180	22.38	1.97	180	29.59	1.87	180
Babia Góra Mt., Marków Stawek	37.95	3.24	264	23.74	2.36	264	29.92	1.68	263

Table 6

Four-level nested ANOVA table for three examined characters of *Trichodina vesicularum* from Poland

Source of variation	Degree of reedom	F_0 — value			Critical value	
		diameter of adhesive disc	diameter of denticulate ring	number of denticles	$F_{0.05}$	$F_{0.01}$
Among groups from various hosts	2–1 = 1	1.316 ns	1.854 ns	2.746 ns	18.51	98.49
Among groups from various altitudes	4–2 = 2	0.313 ns	0.844 ns	3.551 ns	5.79	13.27
Among local groups	9–4 = 5	1.084 ns	1.750 ns	0.837 ns	2.51	3.66
Among particular sub-population	41–9 = 32	25.716 s	19.530 s	13.176 s	1.47	1.71
Within subpopulation	n–41	—	—	—	—	—
Total	n–1					

the 5% level. For the denticulate ring diameter $F_0 = 9.1778$ and for the number of denticles $F_0 = 9.8403$. Simultaneously the differences between particular subpopulations of trichodinas from *T. helveticus* are very high and statistically significant at the 1% level. Especially great differences between subpopulations are observed when the diameter of the adhesive disc is concerned ($F_0 = 57.4881$). In the case of denti-

culate ring diameter $F_0 = 23.0329$, and in the case of the number of denticles $F_0 = 3.6687$.

Thus in the material from Poland as well as from France, the variability is inherent in differences among subpopulations. These differences have probably clonal character.

Among particular subpopulations some differences of qualitative character may also occur. For example in the subpopulation No 103 from *T. montandoni* from the environs of Nowy Targ the ciliates having especially great dimensions of the adhesive disc and greater number of denticles occurred and the means of all other parameters were the greatest or almost the greatest among all examined samples (Table 1). Moreover, silver impregnated preparations revealed an additional, feebly silvered ring around the adhesive disc (Pl. I 9–10) in these ciliates. At present it is difficult to interprete this difference in the pattern of the adhesive disc; probably it is conditioned genetically since it is present in the whole subpopulation.

Within the species *T. vesicularum* fairly great differences may be observed in the shape of denticles in particular specimens, however, their basic character described by K a z u b s k i (1979) is retained. These differences concern the size of denticles and the degree of development of the inner ray (Pl. I 1-10, II 11-14). The outer blade may be longer (Pl. I 5) or narrow (Pl. I 2) and bent near the tip in various modes (cf. Pl. I 7 and 2) but as a rule, the broadest part of the blade is before its mid-length. The inner rays in ciliates from Poland (Pl. I 1-10) as well as in those from lowlands of France (Pl. II 11) are usually shorter than the blades, while in the trichodinas from the Central Massif they are thicker, more solid and at least as long as the blades (Pl. II 12–14). In general, it may be stated that the denticles of trichodinas from the Central Massif are broader and longer than in the ciliates from other localities within the distribution area of *T. vesicularum*.

Trichodina faurefremieti Kazubski, 1979 (Pl. II 15–18)

Trichodina faurefremieti occurs in only one host species — *Triturus cristatus*. For analysis only four subpopulations might have been used, two of them originating from the lowlands (environs of Warszawa) and two from Carpathian highland (environs of Sanok). Mean values of examined characters in samples from these subpopulations are represented in Table 7.

In this material the values of particular characters increase with

Table 7

Mean values (M) and standard deviations (SD) of main characters in

Host	Locality	Altitude above sea level [m]	Date	No. of subpopulation	body		
					M	SD	n
T. cristatus	environs of Warszawa	100	23.05.1964	205	84.85	10.40	21
				207	77.88	11.80	20
	environs of Sanok	350	28.05.1959	137	79.08	9.54	25
				138	77.87	4.76	19
Environs of Warszawa — summarized data					81.45	11.52	41
Environs of Sanok — summarized data					78.56	7.79	44
Summarized data					79.95	9.82	85

the altitude of habitats. Only the body diameter differs in this respect, but reservations to this character have been already discussed.

The analysis of variance, approached to three characters: the diameter of the adhesive disc, the diameter of the denticulate ring and the number of denticles, has shown that the differences dependent on the altitude are statistically insignificant. This result may be due to small number of examined subpopulations. While, similarly as in the case of *T. vesicularum*, the differences among particular subpopulations are significant. For the adhesive disc diameter $F_0 = 3.9893$ being significant at 5% level; for the denticulate ring diameter $F_0 = 6.9883$. These differences are significant at the 1% level.

The differences in the structure of denticles are proportionally small, concerning mainly the length of the inner ray (Pl. II 15–20).

Discussion

Summing up the data on the variability of trichodinas from newts the following conclusions may be drawn. In *T. vesicularum* the geographical variability is observed, being expressed by the change of relations between the denticulate ring diameter and the number of denticles. This variability has been observed in trichodinas from Poland and France, the specimens collected in the Central Massif especially greatly differing in this respect. The host induced variability has not been proved in this species. The trichodinas from *Triturus vulgaris* from the lowland of Poland did not differ virtually from those from

samples from 4 subpopulations of *Trichodina faurefremieti* from Poland

Diameter [μm]									No. of denticles			Length of denticle [μm]			Width of denticle
adhesive disc with border membrane			adhesive disc			denticulate ring									
M	SD	n	M	SD	n	M	SD	n	M	SD	n	M	SD	n	
58.23	3.82	30	49.05	3.57	30	32.52	2.95	30	30.27	1.31	30	17.30	1.48	30	3.37
60.33	5.36	30	51.48	5.09	30	35.12	3.33	30	31.10	1.71	30	17.00	1.82	30	3.55
61.58	7.07	30	52.72	2.88	30	35.34	2.16	29	32.77	1.28	30	17.17	1.89	30	3.39
63.72	3.32	30	53.93	2.95	30	35.52	2.28	30	33.83	1.26	30	18.47	1.95	30	3.30
59.28	4.73	60	50.27	4.53	60	33.82	3.38	60	30.68	1.57	60	17.15	1.66	60	
62.65	5.58	60	53.33	2.95	60	35.43	2.20	59	33.30	1.37	60	17.82	2.01	60	
60.97	5.42	120	51.80	4.11	120	34.62	2.96	119	31.99	1.97	120	17.48	1.87	120	

the lowland of France. Similarly, there were no greater differences between the trichodinas from *T. vulgaris* and those from *T. montandoni* from the Carpathian highland.

The variability connected with the altitude of the habitats above sea level is feebly pronounced in *T. vesicularum*. The differences found in the material from Poland, although conforming with the general tendency to increase body dimensions with the altitude, are too small to be statistically significant. Relatively greater differences were observed in the material from France, however, too small number of examined subpopulations from that country makes this conclusion uncertain.

The variability resulting from belonging to a defined local population does not play a role in *T. vesicularum* and the observed differences are statistically insignificant.

Contrary to this, the variability among particular subpopulations is great, being dominant over the variability induced by any other examined factor. May be, it is connected with relatively great isolation of particular subpopulations. *T. vesicularum* is an inner parasite occurring in the urinary bladder of newts so the exchange of parasites between particular subpopulations is restrained. A supposition may be also made that such subpopulation corresponds to a clone derived from single individual that infests the host. The ciliates from later infections, if any, constitute a small percentage of the subpopulation. Such reasoning elucidates great morphological similarity within particular subpopulations, expressed by small standard deviations of particular characters. An example of such morphological similarity may be the subpopulation No 103 from *Triturus montandoni* from the environs of Nowy Targ,

which almost exclusively comprised large specimens characterized by an additional ring in the adhesive disc.

In the case of *T. faurefremieti,* because of fairly poor material collected, only small variability connected with the altitude of habitats has been observed. It appeared, however, to be statistically insignificant. In this species proportionally great variability among particular subpopulations has been also noted.

When the variability in both species of trichodinas occurring in the urinary bladder of newts is compared great similarity of its character may be seen. In both cases the variability among subpopulations is dominant. It is probably due to their occurrence in the same conditions, in a closed inner organ, to a great degree isolated from outer environment.

The variability of ciliates originating from natural environment was a subject of a number of communications but relatively rarely the phenomenon has been studied in populations, with morphometry based on statistically examined large smaples. So the discussion will be confined to some papers only, more closely related to the phenomena described in the present paper.

The geographical variability has been recently noted in *Ancistrum mytili* from New Brunswick, Canada, Woods Hole, Massachussetts, and San Francisco, California, USA by Berger and Hatzidimitriou (1978) being, however, explained by the differences in host species and local conditions. In the trichodinas from fish such variability was frequently noted, even in the ciliates from the some host species. Those data, however, ought to be once more analyzed because of the possbility that they are influenced by other factors.

More frequently the host induced variability was noted. In the paper mentioned above, Berger and Hatzidimitriou (1978) observed the differences between the ciliates *Ancistrum mytili* from *Mytilus edulis* and *Modiolus modiolus.* Similar differences were noted by Kazubski (1978) in *Myxophyllum steenstrupi* and by Kazubski and Szablewski (1978) in *Tetrahymena rostrata* and *T. limacis* from various snail species.

The variability dependent on environmental factors was studied on the model of *Semitrichodina sphaeronuclea* parasitizing land snail *Bielzia coerulans* (Kazubski 1976). In this trichodina the increase of cell dimensions was observed as well as the adhesive disc diameter and the number of denticles with growing altitude of habitats above sea level. It has been suggested that these changes are due to the decrease of annual mean temperatures characterizing particular habitats on the slopes of the Babia Góra Mt. These differences, verified by Student's

t-distribution test, were statistically significant. Now, these data has been once more analyzed using the analysis of variance and the significance of differences has been ascertained. Thus, it seems that various species of trichodinas may react in different modes against the same environmental factors.

The differences between particular subpopulations were not a frequent object of study. In the paper by B e r g e r and H a t z i d i m i t r i o u (1978) fairly great and statistically important differences have been noted, concerning most parameters of *Ancistrum mytili*. As *Ancistrum* is the representative of *Scuticociliatida* and *Trichodina* of *Peritrichida* it is difficult to make any closer comparisons between them. Nevertheless it seems that in both cases the observed differences are characteristic of the species. Possibly, it is an atribute of parasitic species, forming fairly isolated groups.

ACKNOWLEDGEMENTS

RÉSUMÉ

L'analyse de variance a été utilisé pour l'étude de l'origine de la variation morphologique des Ciliés *Trichodina vesicularum* Fauré-Fremiet et *T. faurefremieti* Kazubski. On a trouvé chez *T. vesicularum* une variation géographique limitée, concernant surtout les individus provenant du Massif Central (France), ainsi qu'une variation limitée liée à l'altitude au-dessus du niveau de la mer. Par contre, la variation entre les différentes souspopulations s'avère importante et significative du point de vue statistique. Ceci paraît être lié ù l'isolation rélativement poussée entre des différentes souspopulations des *Trichodina*, due ù leur mode de vie dans les organes intérieurs de leurs hôtes. La variation entre les souspopulations est également bien exprimée chez *T. faurefremieti*.

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S o k a l R. R. and R o h l f F. J. 1969: Biometry, W. H. Freeman and Co., San Francisco, 776 pp.

EXPLANATION OF PLATES I–II

Trichodina vesicularum Fauré-Fremiet

1–4: specimens from *Triturus vulgaris*
5–8: specimens from *T. montandoni*
9–10: specimens from subpopulation No. 103 of *T. montandoni*
11: specimen from *T. helveticus* (environs of Richelieu, dep. Indre et Loire)
12–14: specimens from *T. helveticus* (environs of Besse-en-Chandesse, dep. Puy de Dôme)

Trichodina faurefremieti Kazubski from *T. cristatus*

15–17: specimens from environs of Warszawa
18–19: specimens from environs of Sanok

Impregnation by AgNO₃. All photographs in the same magnification ca 800 ×

PLATE I

S. L. Kazubski

auctor phot.

PLATE II
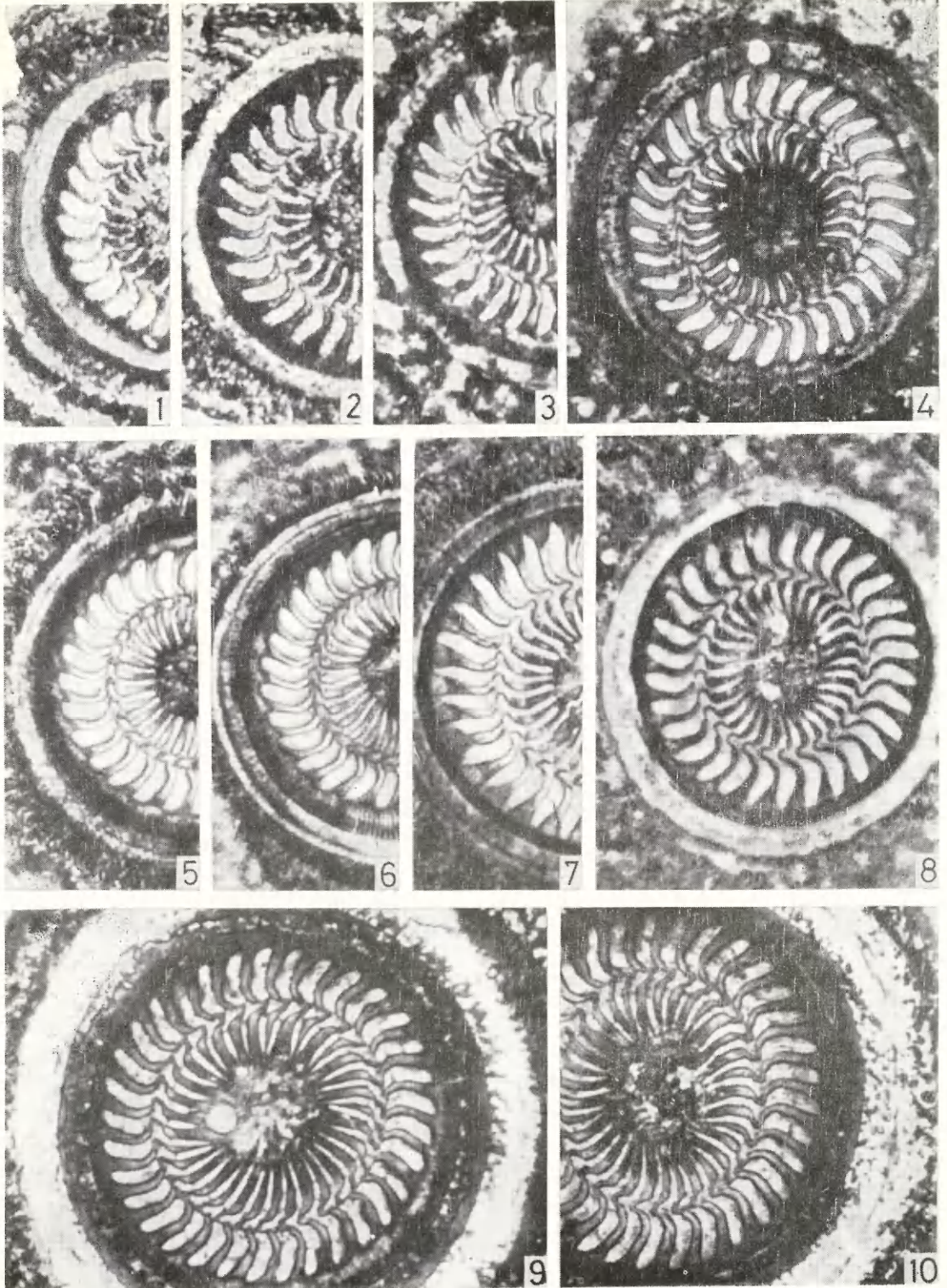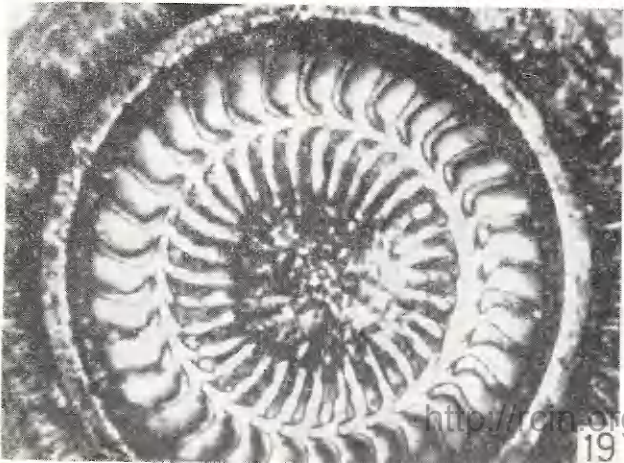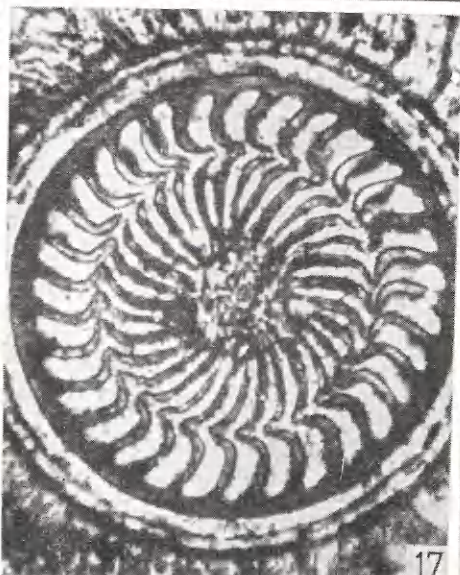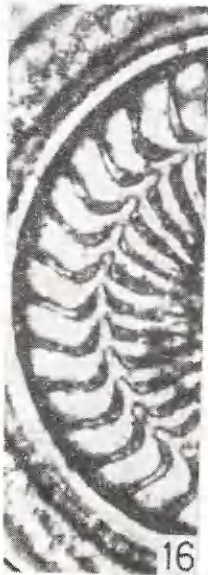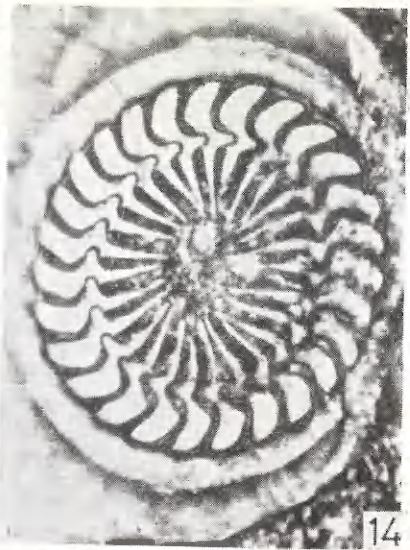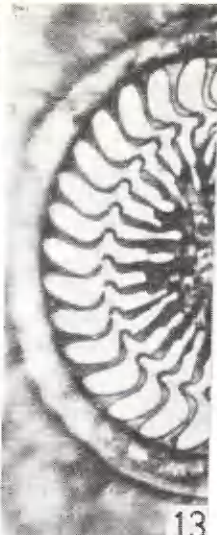
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J e a n   D R A G E S C O   et   A r m e l l e   D R A G E S C O - K E R N E I S

Cilies muscicoles nouveaux ou peu connus

Synopsis. Les auteurs décrivent, à l'aide des techniques d'imprégnation par le Protargol, les espèces de Cilies muscicoles suivantes: *Platyophrya lata* Kahl, *Enchelyodon multinucleata* sp. n., *Spathidium muscorum* sp. n., *Protospathidium muscicola* gen. n., sp. n. L'étude de l'infraciliature buccale des deux *Spathidiidae* montre des stades de complexité croissante dans la disposition des cinéties orales. *P. muscicola* rattache le genre aux Prostomiens plus "primitifs" tandis que *S. muscorum* semblerait s'engager vers la voie Pleurostomienne.

Outre l'observation sur le vivant qui reste, bien entendu, indispensable, nous avons utilisé les techniques de préparation suivantes: l'infraciliature ainsi que la plupart des structures internes (noyaux, toxicystes, nemadesmes, fibres) ou externe (cils, membranelles) ont été mis en évidence par des imprégnations par le Protéinate d'argent (Protargol Roque) suivant deux techniques différentes de fixation: exposition aux vapeurs osmiées et post-fixation par le D u b o s q - B r a s i l (D e r o u x et T u f f r a u 1965, D r a g e s c o et N j i n é 1971) ou fixation directe, en salière, par le Tetraoxyde d'Osmium à 2⁰/₀. La première méthode permet de travailler avec 1 à 6 individus seulement, sans risquer de perdre un seul animal. Elle est rapide, simple économique. La deuxième réussit souvent lorsque les Ciliés refusent de s'imprégner par la première. Elle est plus coûteuse mais très agréable d'emploi car les Ciliés conservent leur forme et leur transparence, n'éclatent jamais et ne collent pas aux sallières et pipettes. L'acide osmique ne nécessite pas de longs lavages avant l'enrobage dans l'albumine glycerinée. L'emploi de la réaction de Feulgen s'est avérée parfois indispensable.

Les Ciliés, fixés et colorés, ont été dessinés à la chambre claire de Wild à des grossissements de 3000 ✕, 2000 ✕ et 1470 ✕. Des photo-

micrographies ont été exécutées au grossissements de 3000 ×. (Ces
divers documents ont permis de procéder à de nombreuses mesures).
La plupart des auteurs publient des mensurations, souvent précises et
analysées par les méthodes de la Biométrie, obtenues sur des Ciliés
fixés et colorés. Malheureusement les processus de fixation, inclusion
et déshydratation entraînent des rétractions, souvent importantes, de
valeur variable et imprévisible. Ces mesures restent par conséquent
assez aléatoires. Il est donc préférable de mesurer les Ciliés sur le
vivant. D'après notre expérience il faut compter sur une rétraction
de 15–30% pour les imprégnations par le nitrate d'argent (Chatton et
Lwoff) et de 25–50% pour les imprégnations par le Protargol (la fixa-
tion à l'acide osmique étant la plus favorable).

Platyophrya lata, Kahl, 1930 (Figs. 1–3, Pl. I 1, 2)

La cellule ressemble à une petite bourse très aplatie dont l'ouver-
ture, déjetée sur le côté, est assez large. A première vue ce Cilié, trans-
parent et relativement lent, rappelle un *Spathidium*. De forme assez
variable (Fig. 1) sa longueur varie sur le vivant, entre 80 et 120 μm.
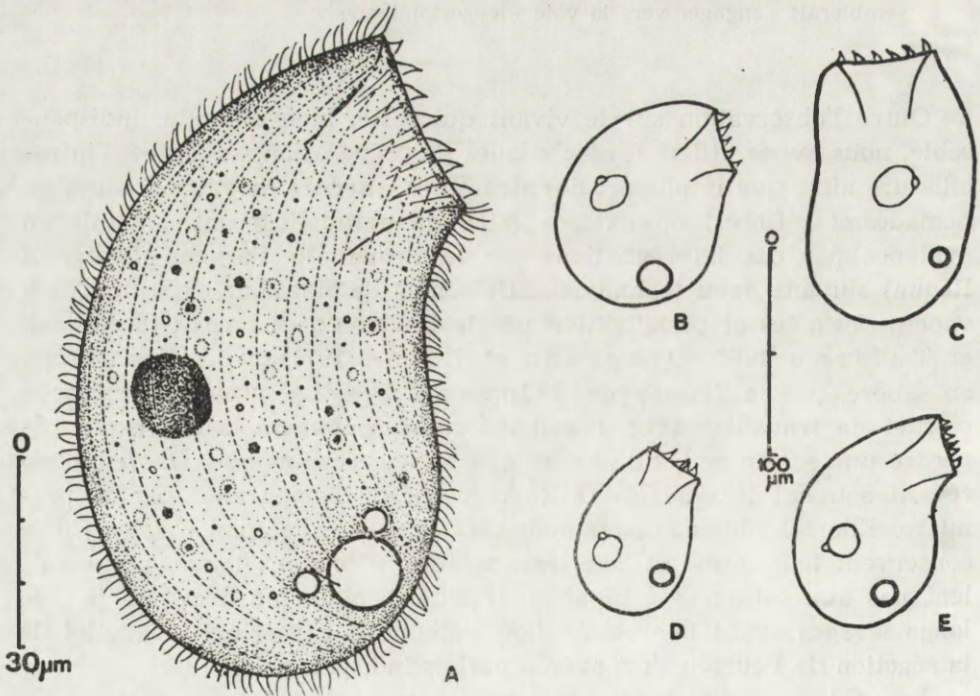
Fig. 1. *Platyophrya lata* Kahl: A — aspect général sur le vivant, B–E — variation
de forme et de taille (sur le vivant)

Après fixation, les dimensions suivantes ont été mesurées: longueur de 45 à 88 μm (moyenne 70 μm), largeur de 34 à 58 μm (moyenne 46.5 μm). Les cils mesurent 4 μm de long et leur écartement est de 1.4 μm, en moyenne. Contrairement à *Platyophrya spumacola* l'espèce *P. lata* n'est pas très métabolique. Les cinéties se trouvent disposées sur des crêtes assez nettement perceptibles. La bouche est apicale, plus ou moins déjetée sur le côté (ventral ?). La ciliature somatique est constituée par environ 33 cinéties bi-polaires, disposées en spirale, plus prononcée que chez *P. spumacola,* sur le côté droit (leur nombre varie de 18 à 22). La longueur de ces cinéties est très inégale: elles décrivent, comme chez *P. spumacola,* une ligne de sécance au voisinage du pore de la vésicule contractile (Fig. 2). Sur la face gauche, les cinéties sont
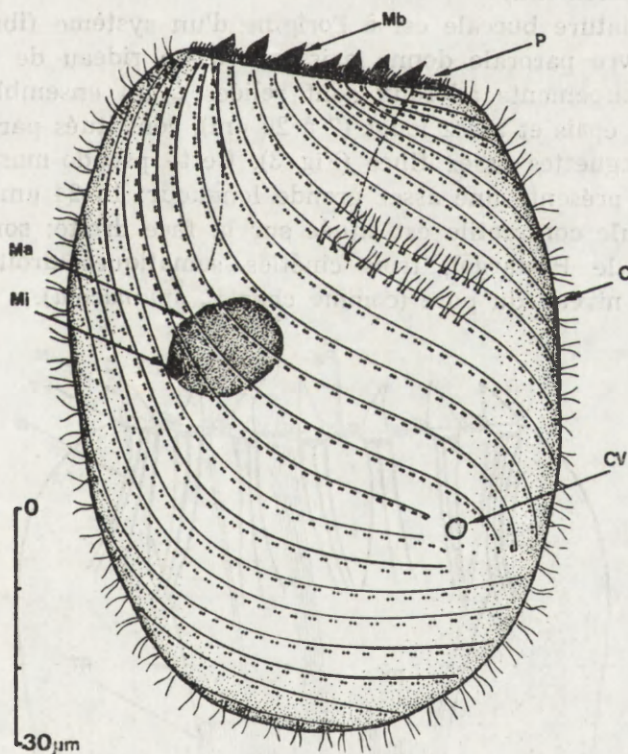
Fig. 2. *Platyophrya lata* Kahl: Vue générale de l'infraciliature droite d'après une imprégnation par le Protargol (dessin à la chambre claire). *Légende*: MB —"membranelles", P — cinétie "pararole", C — cils somatiques, CV — vésicule contractile, Ma — macronoyau, Mi — micronoyau

peu spiralées, presque bi-polaire. Leur nombre varie de 17 à 18 seulement (leur espâcement est donc plus grand). Les cinétosomes sont doublés par une forte fibrille argyrophile.

La ciliature buccale est du même type que chez *P. spumacola*: la fente buccale allongée mesure de 16 à 20 µm, elle est bordée, à droite, par une cinétie parorale, à cinétosomes très serrés, porteurs de cils très courts (vu le fort aplatissement du Cilié il nous est impossible de préciser si la parolale est double ou simple). A gauche de l'ouverture buccale on observe un "ensemble adoral", composé de 6 à 8 groupes de cinétosomes serrés, constituant de sortes de "pavés" allongés qui sont le point de départ de 6 à 8 "membranelles". Contrairement à ce qui a été décrit chez *P. spumacola* (G r o l i è r e 1975, D r a g e s c o et al. 1977) les "paves" semblent plutôt parallèles à la fente buccale. Chaque groupe adoral serait constitué par une double rangée d'au moins 6 cinétosomes chacun. (Chez *P. spumacola* les mêmes groupements présentent 3 × 2 cinétosomes seulement.)

L'infraciliature buccale est à l'origine d'un système fibrillaire complexe: la lèvre parorale donne naissance à un rideau de tubules très fins; les groupements adoraux sont reliées à un ensemble de 6 à 8 némadesmes épais et assez longs (7 à 20 µm), constitués par un faisceau de 2 à 4 baguettes assez fines (Fig 3). Cette pseudo-masse (sorte de "clathrum") présente une assez grande longueur: 15–24 µm.

La vésicule contractile est située sur la face droite; son pore s'imprègne par le Protargol, trois cinéties somatiques droites viennent s'arrêter au niveau du pore (comme chez *P. spumacola*).
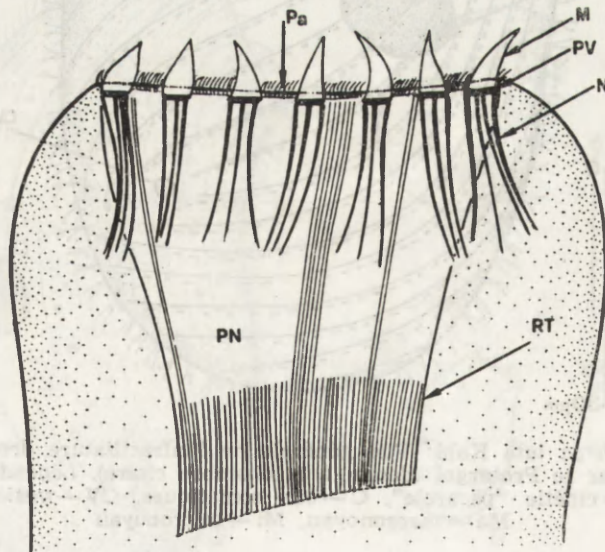
Fig. 3. *Platyophrya lata* Kahl: Schéma des détails des armatures fibrillaires buccales (d'après des imprégnations par le Protargol). *Légende*: Pa — "parorale", M — "membranelle", PV — "pavé", N — némadesme, PN — pseudonasse (clathrum), RT — rideau de tubules

L'appareil nucléaire est constitué par un macronoyau sphéroïde de 9 à 16 μm de diamètre (moyenne: 12 μm) et par un micronoyau lenticulaire, étroitement appliqué contre le macronoyau. L'ensemble de l'appareil nucléaire est entouré par une membrane unique (comme chez les autres *Platyophrya*).

Platyophrya lata se nourrit de Colpodes et de Tétrahymeniens. De cette étude, quelque peu incomplète, nous concluerons que *P. lata* montre le même type de structure que *P. spumacola* Kahl, *P. viridis* (Gelei) et *P. angusta* Kahl (D r a g e s c o et al. 1977, G r o l i è r e 1976).

Enchelyodon multinucleata sp. n. (Figs. 4, 5, Pl. I 3–5)

En forme de bouteille légèrement aplatie, le goulot déjeté sur le côté, cet Infusoire, grand prédateur de Colpodes, mesure 180 à 180 μm, sur le vivant (les individus fixés ont une longueur de 90 à 140 μm, moyenne 116 μm et une largeur de 58 à 62 μm, moyenne 60 μm) (Fig. 5 A).

L'infraciliature est constituée de 34 cineties bi-polaires (en moyenne). La ciliature somatique s'arrête un peu avant l'ouverture buccale, apicale, découvrant ainsi une petite calotte glabre, sorte de mucron, visible surtout du côté dorsal (Fig. 4 A). Les cinéties se spiralisent nettement, du côté ventral, et leurs cinétosomes sont légèrement plus denses au voisinage de l'ouverture buccale. Du côté dorsal cette spiralisation est beaucoup moins apparente on y observe aussi une "structure en brosse" typique, d'aspect quelque peu variable. Elle intéresse généralement trois cinéties de 12 μm de longueur maximum. La cinétie la plus longue, de la brosse, compte de 21 à 22 couples de cinétosomes serrés qui semblent reliés, à leur base, par une fibrille argyrophile. De la plupart de ces cinétosomes spécialisés se dresse une très courte sois raide.

Autour de l'ouverture buccale, en forme d'entonnoir dissymétrique (Fig. 4 C), on colore parfois des toxicystes assez courts (2.5 μm) dont on observe, dans le cytoplasme, divers stades de gênèse.

De longues némadesmes forment un "panier" lâche.

La vésicule contractile postérieure est d'assez grande taille. Au moment de la diastole il se forme des vacuoles satellites. L'appareil nucléaires, très caractéristique, est constitué par plus de 100 macronoyaux sphéroïdaux, ou le plus souvent, allongés, mesurant de 1.5 à 3.5 μm. Parmis eux on observe aussi de 20 à 30 micronoyaux sphériques de 2.5 μm de diamètre.

Au moment de la division les cinétosomes de la région équatoriale se multiplient, les cinéties se coupent et se courbent au niveau de la
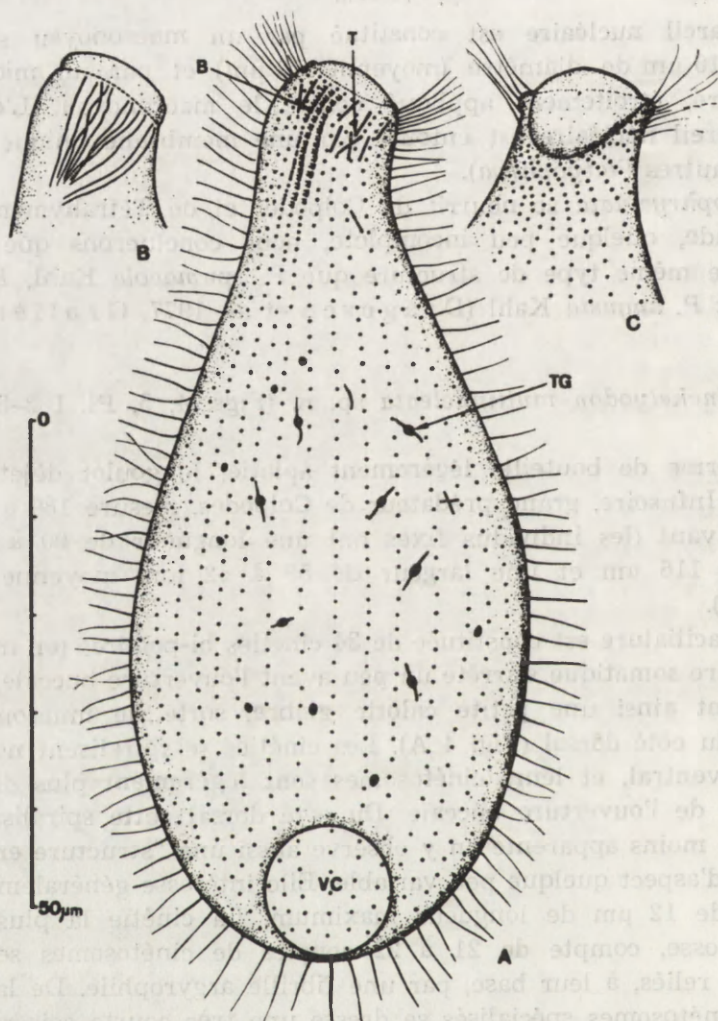
Fig. 4. *Enchelyodon multinucleata* sp. n. (dessin à la chambre claire, d'après des imprégnations par le Protargol), A — aspect général de l'infraciliature, B — némadesmes, C — ciliature péribuccale ventrale. *Legende*: B — "structure en brosse", T — toxicystes, TG — toxicystes genèse, VC — vésicule contractile

future bouche de l'optisthe, pendant que les macronoyaux deviennent de longs rubans dustiles et fibreaux qui envahissent toute la cellule. (Les micronoyaux entrent au mitose plus tardivement (Fig. 5 C et E).

La détermination de ce Cilié nous a présenté des difficultés. Son éminence buccale glabre donne à penser un représentant du genre *Enchelyodon*, mais l'appareil nucléaire le différencie très nettement de tous les autres *Enchelyodon* déjà décrits. L'infraciliature buccale
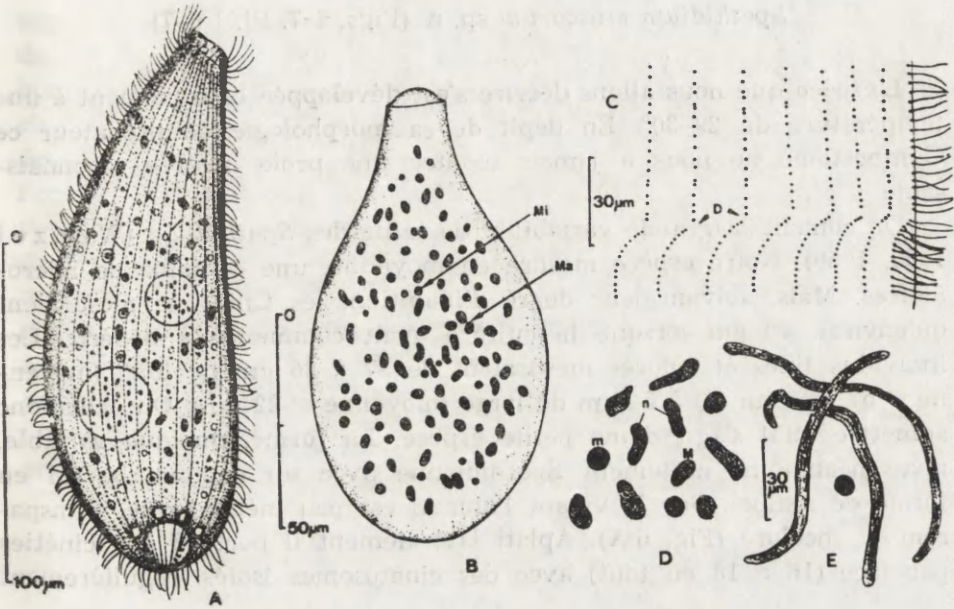
Fig. 5. *Enchelyodon multinucleata* sp. n. A — aspect sur le vivant, B — appareil nucléaire (Protargol), C — comportement des cinéties somatiques au moment de la division (Protargol), D — détail de l'appareil nucléaire (Protargol), E — allongement des macronoyaux à la division. *Légende*: Ma — macronoyau, Mi — micronoyau, D — zone de division, M — macronoyau, Mi — micronoyau, n — nucléole

de la plupart des autres espèces restant encore inconnue les comparaisons sont malaisées. B o r r o r (1965) sur *E. trepida* (Kahl) représente des cinéties peribuccales plutôt convergentes et une "structure en brosse" constituée d'une seule cinétie. G r o l i è r e (1976) sur *E. sphagni* figure des cinéties rectilignes, dont seulement une seule est transformée en "structure en brosse". D r a g e s c o (1970) sur *E. vermiformis* semble avoir vu l'extrémité des cinéties somatiques recourbées vers la droite mais ses figures restent ambigues, l'imprégnation de cette espèce ayant été très médiocre. Cette forte incurvation des extrémités des cinéties, dans la zône orale, nous permet de rapprocher notre espèce du genre *Chaenea*, cilies très allongées, souples et métaboliques. Un rapprochement peut être tenté avec le genre *Enchelys* mais l'"infraciliature buccale de *E. pellucida* (D r a g e s c o et al. 1974) se montre différente: les cinétosomes sont beaucoup plus denses dans la zône peribuccale et les cinéties restent parfaitement rectilignes.

Dans l'attente d'une meilleure connaissance de ces genres voisins nous appelerons notre cilié *Enchelyodon multinucleata* sp. n., caractérisé par son appareil macronucléaire pulvérisé.

Spathidium muscorum sp. n. (Figs. 6-7, Pl. I 6-7)

L'espèce que nous allons décrire s'est développée brusquement à une température de 29–30°. En dépit de sa morphologie de prédateur ce Gymnostome ne nous a jamais montré une proie ingérée reconnaissable.

On connaît la grande variabilité de taille des *Spathidium* (W e n z e l 1955, 1959). Notre espèce mesure en moyenne, une centaine de micromètres. Mais, suivant leur degré d'inanition, les Ciliés ne mesuraient qu'environ 40 um lorsque la culture avait commencé à dépérir. Les individus fixés et colorés mesuraient de 37 à 86 µm de long (moyenne = 57 µm) sur 10 à 54 µm de large (moyenne = 22 um). On peut donc admettre qu'il s'agit d'une petite espèce. La forme, quoique variable, reste néanmoins nettement Spathidienne avec un contour buccal en forme de scalpel. Sur le vivant l'animal est peu métabolique, transparent et incoloré (Fig. 6 A). Aplati latéralement il porte 8 à 9 cinéties par face (16 à 18 en tout) avec des cinétosomes isolés, régulièrement
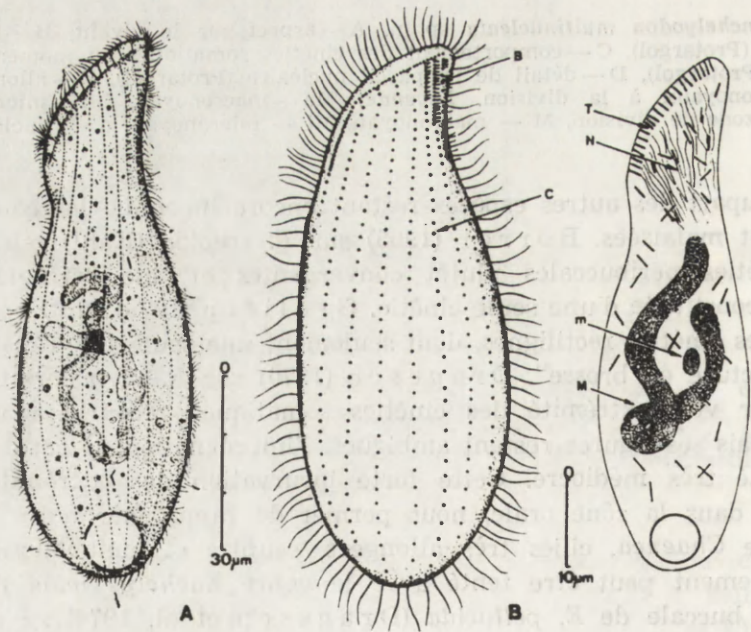
Fig. 6. *Spathidium muscorum* sp. n. A — aspect général sur le vivant, B — infraciliature (dessin à la chambre claire, d'après une imprégnation par le Protargol), C — structure interne (image synthétique, d'après des imprégnations par le Protargol et des réactions de Feulgen). *Légende*: B — "structure en brosse", C — cinétosomes somatiques, T — toxicyste, N — némadesnes, M — macronoyau, m — micronoyau

espacés, générateurs de cils, longs de 5 à 7 µm. Légèrement au-dessous de l'ouverture buccale (qui est une fente virtuelle représentée par le bord externe du "scalpel") les cinéties somatiques, bi-polaires, s'infléchissent du côté ventral sur la face gauche et du côté dorsal sur la face droite (Fig. 6 B et 7 A et B). Comme F r y d - V e r s a v e l et al. (1975) l'on montre sur trois espèces, les cinéties somatiques s'infléchissent, ventralement et dorsalement, de manière à venir constituer une "pseudo-cinétie" continue circumorale. B u i t k a m p (1977) sur *Spathidium muscicola* Kahl montre, très clairement, le rebroussement brutal des cinéties somatiques qui, au niveau de la lèvre buccale, se courbent à 90° et donnent l'impression d'une cinétie continue (l'effet est beaucoup moins net sur le côté droit, comme chez les autres espèces connues).

Chez *Spathidium muscorum* l'illusion de la cinétie circumorale est encore plus grande (Fig. 7 A, B et C), car les cinéties sont moins nom-
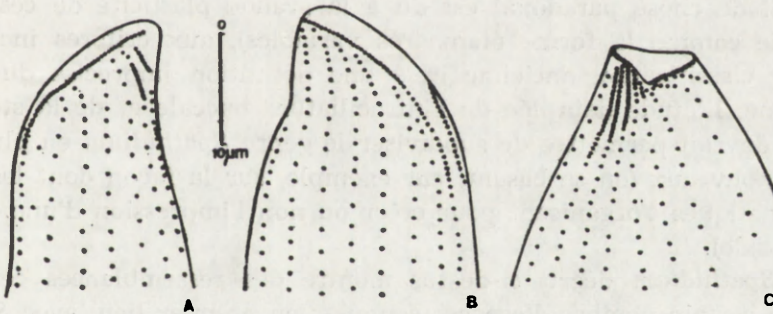
Fig. 7. *Spathidium muscorum* sp. n. Infraciliature de la région buccale (d'après des imprégnations par le Protargol) A — côté gauche (montrant aussi "la structure en brosse", B — côté droit (montrant la cinétie d'apparence circum-orale), face dorsale montrant la "structure en brosse" et le recourbement des extrémités antérieures des cinéties somatiques

breuses et les cinétosomes peu serrés (sur les extrémités recourbées). Il y a non seulement infléchissement du côté ventral, mais aussi forte prolifération cinétosomienne au niveau des fragments antérieurs qui se sont mis bout à bout. Du côté droit, l'inclinaison des cinéties étant plus faible, l'illusion d'une cinétie peribuccale indépendante est encore plus forte (contrairement à ce que décrit B u i t k a m p 1977, sur *S. muscicola* où les cinéties droites semblent se recouvrir tout simplement).

Dans la région antéro-dorsale il existe une "structure en brosse", constituée de 3 à 4 courtes cinéties, avec de cinétosomes simples, très serrés, porteurs de courtes soies raides (Fig. 6 A et 7 C). Si la connaissance précise de l'infraciliature buccale des *Spathidium* est une acquisition très récente, nous devons rappeler que, même avant l'utilisa-

tion du Protargol, des anciens auteurs avaient bien compris la courbure des portions apicales des cinéties somatiques au niveau de la bouche ainsi que la "structure en brosse": L u k a c s (1935), G e l l e r t (1955 et 1956).

Spathidium muscorum montre, par ailleurs, une structure tout à fait classique. Les lèvres buccales sont garnies de fins toxicystes de 2 µm de long et de némadesmes longs et flexueux. Le macronoyau est un épais boudin contourné et le micronoyau unique, lenticulaire, est très volumineux (ϕ = 3.5–5 µm). Le rapport nucléo-plasmique est très élevé, comme chez la plupart des *Spathidium*. La vésicule contractile est, bien entendu, terminale.

Le problème de la détermination des *Spathidium* est l'un des plus ardus que nous connaissons. D'après nos évaluations, 110 espèces de *Spathidium* ont déjà été décrites. D'autre part, chaque fois qu'un auteur moderne étudie des représentants du genre, il en découvre de nouveaux. Cet état de chose paradoxal est du à la grande plasticité de ces Ciliés (la taille comme la forme étant très variables), aux critères incertains de leur classification ancienne et à une définition imprécise du genre lui-même. L'étude détaillée de l'infraciliature buccale et de la stomato-génèse devrait permettre de subdiviser le genre *Spathidium* en plusieurs genres nouveaux (en se basant, par exemple, sur la façon dont les cinéties somatiques s'organisent pour créer ou non l'impression d'une cinétie circumorale).

Le *Spathidium* décrit ci-dessus montre des ressemblances certaines avec un certain nombre d'espèces connues: en premier lieu, avec *S. scalpriforme*, dont il présente la forme, en scalpel, de la région buccale. Mais *S. scalpriforme* est de trois à quatre fois plus grand, présente un grand nombre de petits micronoyaux, d'avantage de cinéties et des toxicystes plus longs. *Spathidium muscicola* (variante de K a h l de 1931, Fig. 2, p. 164) rappelle beaucoup notre espèce lorsqu'elle est en état d'inanition prolongée mais il est normalement deux à trois fois plus grand, ses toxicystes sont énormes, les cinéties et les micronoyaux nombreux. Remarquons que ces deux espèces sont muscicoles aussi et dérivent peut être d'une forme synthétique commune.

On pourrait aussi faire des rapprochements avec *Spathidium spathula* (O.F.M.) ont tout au moins la variante trouvée par W e n z e l (1953). Mais ce Cilié est amicronuclée, son macronoyau est plus ramassé et la forme de la région buccale différente. En fait, faute de connaître l'infraciliature buccale de ces trois espèces voisines on ne peut assimiler la nôtre à aucune d'elles, d'autant plus que nous connaissons très mal la variabilité des divers caractères envisagés. Une assimilation arbitraire ne nous satisfaisant pas d'avantage, nous préférons donc créer une espèce nouvelle qui sera

désormais un peu mieux définie. *S. muscorum* est donc une petite espèce, d'une soixantaine de micromètres, dont la région buccale présente une forme caractéristique (en scalpel) et qui se définit encore par ses 18 cinéties, ses courts toxicystes et, surtout, son infraciliature orale: cinétie d'apparence circumorale semblant indépendante des cinéties somatiques.

Protospathidium muscicola gen. n., sp. n. (Figs. 8, 9, Pl. I 8–9)

Un petit Gymnostome s'est rapidement développé en assez grand nombre, à la température de 30°. De forme allongée, presque nématomorphe, sa longueur varie (sur animaux fixés et colorés) de 52 à 110 µm (moyenne 75.2 µm), tandis que sa largeur ne dépasse pas 10 à 18 µm (moyenne 13 µm). Sur le vivant, il mesure jusqu'a 140 µm et son rapport longueur/
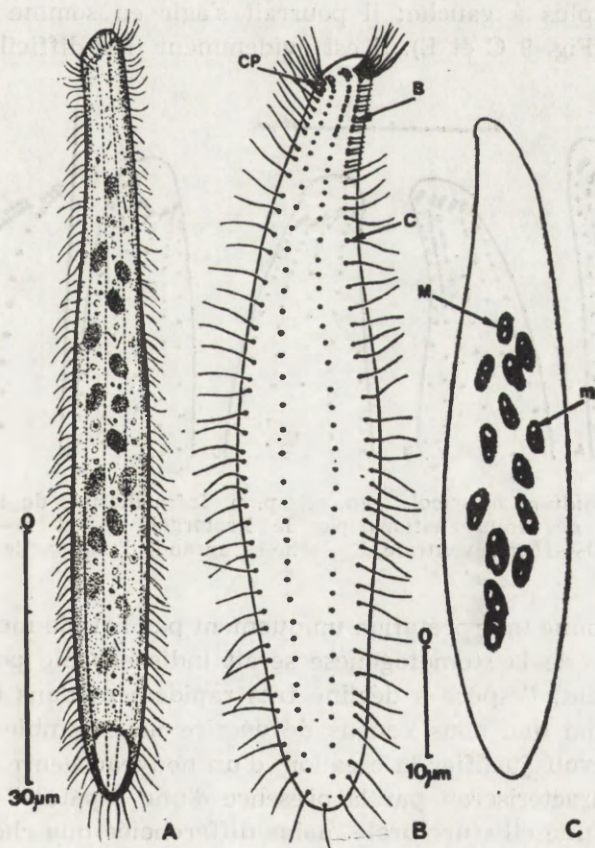
Fig. 8. *Protospathidium muscicola* gen. n., sp. n. A — aspect général sur le vivant, B — infraciliature générale (côté gauche) — dessin à la chambre claire, d'après une imprégnation par le Protargol, C — appareil nucléaire (réaction de Feulgen), *Légende*: CP. — cils peribuccaux, C — cinétosomes somatiques, B — "structure en brosse", M — macronoyau, m — micronoyau

/largeur est plus faible. Transparent et incoloré, ce Gymnostome est un prédateur vorace de petits Flagellés. Les cils assez longs (4.5 µm) sont disposés sur 10 à 12 cinéties bi-polaires régulièrement espacées (avec environ 40 cinétosomes par cinétie) (Fig. 8 A et B).

Les cinéties somatiques se courbent, un peu en avant de la petite spatule buccale. Elles se dirigent alors ventralement sur le côté gauche, dorsalement sur le côté droit (comme chez les *Spathidium*). Chaque extrémité apicale, ainsi courbée se montre constituée par une double rangées de cinétosomes: en moyenne six cinétosomes contiguës, en continuité avec la cinétie somatique respective et 4 autres, tout aussi serrés, au-dessus des premiers. Certaines images donnent l'impression d'un simple dédoublement des cinétosomes apicaux (Fig. 9 A, B et D), d'autres font penser que le fragment supérieur n'est que la continuité de la cinétie somatique qui se trouve plus à gauche; il pourrait s'agir en somme d'un simple recouvrement (Fig. 9 C et E). Il est évidemment très difficile de décider
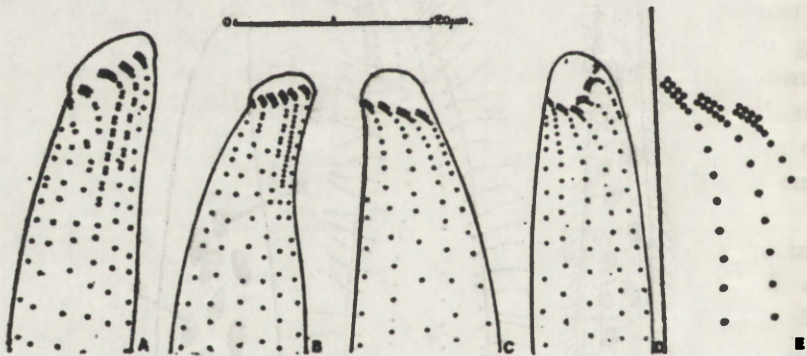
Fig. 9. *Protospathidium muscicola* gen. n., sp. n. Infraciliature de la région péribuccale (d'après des imprégnations par le Protargol), A et B — face gauche, C — face droite, D — région ventrale, E — schéma agrandi d'une partie de la figure C

qu'elle est la bonne interprétation uniquement par l'étude morphologique; la connaissance de la stomatogénèse serait indispensable pour conclure. Malheureusement, l'espèce a décliné très rapidement dans nos cultures.

La disposition que nous venons de décrire nous semble assez particulière pour devoir justifier la création, d'un nouveau genre *Protospathidium* qui se caractériserait par la présence d'une "spatule" buccale peu apparente et d'une ciliature orale moins différenciée que chez la *Spathidium* "sensu stricto". Le nouveau genre se différencie des *Enchelyodon* par une torsion plus grande des segments apicaux des cinéties somatiques et des *Spathidium* par l'absence d'une apparence de cinétie périorale (ou circum-orale).

P. muscicola possède une "structure en brosse" constitué, généralement, par 2 cinéties dont les cinétosomes sont plus resserrés et groupés en doublets. Il peut arriver aussi que l'une de ces cinéties soit constituée par des cinétosomes serrés régulièrement disposés. Les cinétosomes de la "brosse" portent des soies assez longues (Fig. 8 et 9 A et B). Les toxicystes sont longs aussi (plus de 3.5 µm) et assez fins. On note encore une vésicule contractile terminale et un appareil nucléaire important, constitué essentiellement par 10 à 20 macronoyaux assez volumineux (3 à 4 µm de longueur). Nous n'avons pas pu observer de micronoyau. Le genre est donc représenté, pour le moment, par l'espèce type que nous venons de décrire. *P. muscicola* ressemble à deux *Spathidium* déjà décrits:

(1) à *Spathidium serpens* Kahl, 1931, dont il possède la forme nématomorphe et le nombre de cinéties (Mais si *S. serpens* présente une taille équivalente, il se distingue de notre espèce par une spatule plus développée et un macronoyau en chapelet);

(2) à *Spathidium bonneti* Buitkamp, 1977, mais ce dernier est plus grand et plus filiforme, ses cils sont plus longs et beaucoup moins denses, sa spatule plus large et son infraciliature buccale différente (remarquons que l'infraciliature de *S. bonneti* est assez peu orthodoxe, si on se base sur la figure, pas très visible, de l'auteur allemand). *Spathidium anguilla* Vuxanovici, 1962, présente aussi une certaine ressemblance de forme et une longueur du même ordre, mais la description de l'auteur roumain est trop sommaire pour permettre une comparaison efficace. Toutes les autres 107 espèces de *Spathidium* connues ne peuvent évoquer de ressemblance avec notre Cilié. Nous admettons donc qu'il s'agit bien d'une espèce nouvelle caractérisée par sa forme allongée, sa petite spatule, son appareil nucléaire dispersé et la curieuse disposition de ses cinéties orales.

Discussion et conclusions

Les Ciliés Gymnostomes ont une très grande importance dans tout essai d'analyse évolutive et phylogénétique du groupe tout entier. Ce sont, malheureusement, des Infusoires encore très mal connus. Il est donc intéressant d'essayer de discuter des affinités structurales de certaines espèces que nous venons de passer en revue.

Enchelyodon multinucleatum nous démontre l'incertitude de nos connaissances sur l'infraciliature buccale de tout un groupe de Ciliés Gymnostome "primitifs" (*Chenea, Enchelys, Enchelyodon*). Les *Spathidiidae* représentent un grand ensemble encore très mal connu (en dépit des récents travaux qui lui ont été consacrés). Notre nouveau genre *Protospathidium*

muscicola pourrait être considéré comme une forme relativement primitive car les cinéties somatiques s'incurvent simplement, dans leur portion apicale, sans donner l'apparence d'une ciliature circum-orale spécialisée (comme chez les *Spathidium* "*sensu stricto*" (Fig. 10 A). Cette cliature
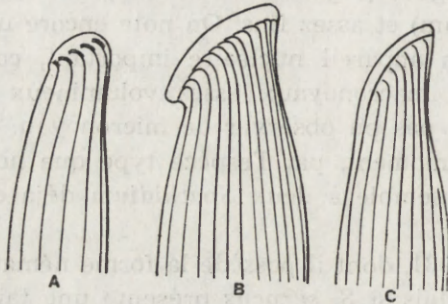
Fig. 10. Tendances évolutives possibles chez les *Spathiidae*: disposition schématique de l'infraciliature péri-buccale, A — type *Protospathidium*, B — ype *Spathidium sensu stricto*, C — type évolué (*S. muscorum*). Détails dans le texte)

buccale rappelle plutôt celles qu'on a pu décrire chez des Gymnostomes prostomiens plus "primitifs" (tels *Chaenea* ou *Enchelyodon*). Plus troublant est le dédoublement des segments, tout à fait apicaux, de portions recourbées des cinéties, au niveau même de l'ouverture buccale. Les *Spathidium* "sensu stricto" montrent, sous des aspects légèrement variables, l'apparence d'une cinétie circum-orale, visible surtout sur le côté gauche, constitué en fait par les extrémités des cinéties somatiques recourbés, se rejoignant de manière à donner une illusion de continuité (Fig. 10 B).

Spathidium muscorum, qui appartient peut-être à un genre nouveau, représenterait un état nettement plus "evolué". Ce Cilié semble avoir réalisé une cinétie réellement circum-orale, indépendante des cinéties somatiques (Fig. 10 C). Son rattachement aux portions apicales des cinéties somatiques reste parfois encore perceptible. On observe, en outre, que cette cinétie est interrompue aussi bien ventralement que dorsalement (Fig. 7 B et C) de sorte qu'il s'agit pratiquement de deux cinétie peribuccales (droite et gauche). On serait tenté de penser que *S. muscorum* pourrait montrer la voie d'une évolution entraînant les Gymnostones sur la voie Pleurostomienne.

L'étude détaillée de l'infraciliature et de la stomatogénèse de diverses espèces de *Spathidium* devrait permettre de découvrir d'autres niveaux évolutifs interéssants.

SUMMARY

The authors describe (with the help of the protargol silver impregnation techniques) the following species of moos ciliates *Platyophrya lata* Kahl, *Enchelyodon multinucleata*, sp. n., *Spathidium muscorum* sp. n., *Protospathidium muscicola* gen. n., sp. n.

The study of the mouth ciliation of the two *Spathidiidae* shows growing stages of complexity in the arrangement of the oral kineties: *P. muscicola* links gender *Spathidium* to the more primitive *Prostomata* whereas *S. muscorum* seems to suggest a possible evolution towards the *Pleurostomata*.

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EXPLANATION DE PLANCHE I

1: *Platyophrya lata*: infraciliature (Protargol)
2: *Platyophrya lata*: armature buccale (némadesmes et rideau de tubules) (Protargol)
3: *Enchelyodon multinucleata*: ciliature buccale ventrale (Protargol)
4: *Enchelyodon multinucleata*: "structure en brosse" (Protargol)
5: *Enchelyodon multinucleata*: macronoyaux et micronoyaux (Protargol)
6: *Spathidium muscorum*: infraciliature buccale gauche (Protargol)
7: *Spathidium muscorum*: infraciliature dorsale (et "structure en brosse")
8: *Protospathidium muscicola*: infraciliature buccale gauche et "structure en brosse"
(Protargol)
9: *Protospathidium muscicola*: infraciliature buccale ventrale (Protargol)

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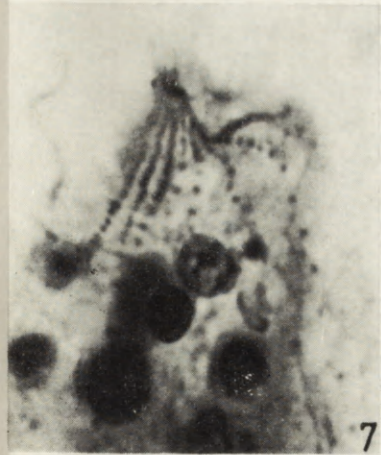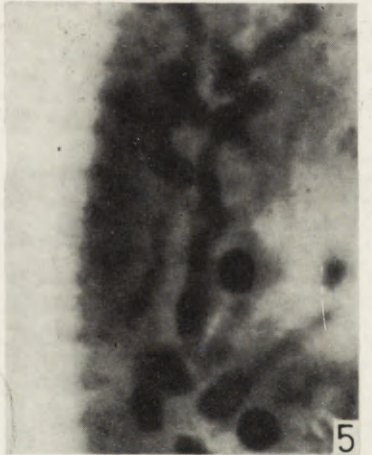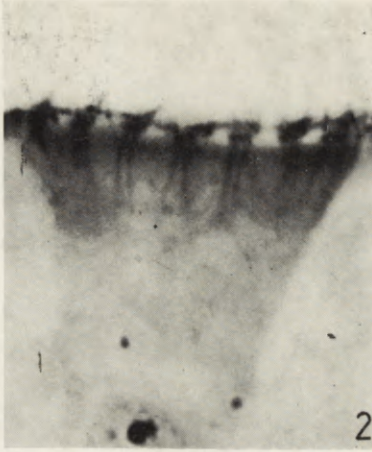
Wilhelm FOISSNER

Taxonomische Studien über die Ciliaten des Grossglocknergebietes (Hohe Tauern, Österreich). III. Familien *Tracheliidae, Didiniidae, Nassulopsidae* und *Orthodonellidae*

Synopsis. Es wird die Morphologie, die Infraciliatur und das Silberliniensystem einiger Ciliaten (*Dileptus anser. Dileptus visscheri, Acropisthium mutabile, Nassulopsis paucivacuolata* sp. n., *Chilodontopsis depressa*) des Großglocknergebietes (Hohe Tauern, Österreich) beschrieben. Die systematische Stellung der Genera *Nassulopsis* und *Chilodontopsis* wird kurz diskutiert. *Nassulopsis* weist Beziehungen zu den *Synhymeniida* und *Nassulida* auf. *Chilodontopsis* ist ein typischer synhymenider Ciliat mit einem besonders differenzierten Silberliniensystem.

Im dritten Teil meiner Monographie (F o i s s n e r 1979 a,b, 1980) über die Ciliaten der Kleingewässer entlang der Großglockner-Hochalpenstraße wird je ein Vertreter der *Tracheliidae, Didiniidae, Nassulopsidae* und *Orthodonellidae* beschrieben. Besonderes Gewicht wird auf das Silberliniensystem gelegt, da es zur Abklärung der systematischen Stellung einiger problematischer Genera beitragen kann.

Material und Methoden

Das Untersuchungsmaterial wurde in den Kleingewässern (Weidetümpeln, Schmelzwassertümpeln etc.) entlang der Großglockner-Hochalpenstraße gesammelt. Genauere Fundortangaben und ökologische Daten finden sich bei F o i s s n e r (1979 c). Darauf bezieht sich auch die Numerierung des Tümpels beim Locus typicus von *Nassulopsis paucivacuolata* nov. spec.

Zur Darstellung der Infraciliatur und des Silberliniensystems verwendete ich die nasse Silberimprägnationsmethode von C o r l i s s (1953) und die trockene Silberimprägnationsmethode von F o i s s n e r (1976). Der Kernapparat wurde mit Orcein-Essigsäure angefärbt. Alle Arten wurden einer genauen Lebendbeobachtung unterzogen.

Beschreibung der Arten

(1) Familie *Tracheliidae* Ehrenberg

Dileptus anser Müller, 1786 und Dileptus visscheri Dragesco, 1963
(Abb. 1, Taf. I 5–7)

Morphologie und Diskussion: Diese zwei Arten entsprachen der Darstellung von D r a g e s c o (1963). Deswegen wird nur das Silberliniensystem besprochen, über das lediglich eine kurze Beschreibung von K l e i n (1930) existiert. Es ist ein linear orientiertes Engmaschengitter, das sich kontinuierlich über die ganze Zelle ausbreitet (Abb. 1).
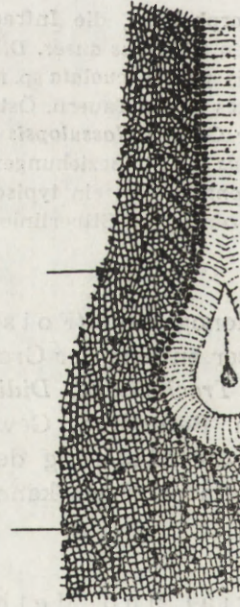
Abb. 1. *Dileptus anser.* Silberliniensystem und Infraciliatur im Bereich des Oralapparates. Die Pfeile weisen auf Relationskörper der Protrichocysten. Trockene Silberimprägnation

Im Bereich des Rüssels und Schwanzes (Taf. I 6) ist es ziemlich unregelmäßig. Im mittlerer Körperabschnitt ist das Gitter schräg zu den Kineten orientiert (Taf. I 5, 7). In den Gitterstoßpunkten liegen die Relationskörper der Protrichocysten (Abb. 1, Taf. I 5).

(2) Familie *Didiniidae* Poche
Acropisthium mutabile Perty, 1852 (Abb. 2, Taf. I 8, 9)

M o r p h o l o g i e und D i s k u s s i o n : Der Vergleich der Arbeiten von P e r t y (1852), E b e r h a r d (1858, 1862), B ü t s c h l i (1887–89), S c h e w i a k o f f (1889), R i m s k y - K o r s a k o w (1897), K a h l (1926) und B o h a t i e r et al. (1973) sowie eigene Beobachtungen überzeugten mich davon, daß K a h l (1930–35) mit Recht nur eine Art in dieser Gattung gelten ließ. Schlecht ernährte Individuen sind rübenförmig (Abb. 2), gut ernährte tropfenförmig. Die 45–63 µm großen Tiere sind sehr gefräßig; oft findet man 10 Nahrungsvakuolen mit *Chilomonas* sp. Kontraktile Vakuole und Cytopyge münden subterminal aus (Abb. 2). Die Infra-

2
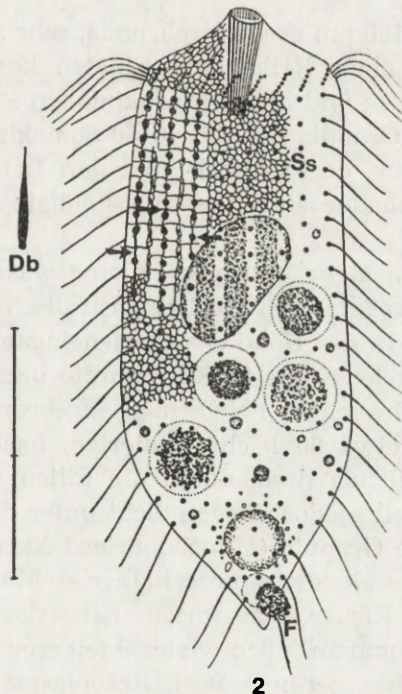
Abb. 2. *Acropisthium mutabile*. Infraciliatur und Silberliniensystem (Ss) nach trockener Silberimprägnation. Körperform und Dorsalborste (Db) nach Lebendbeobachtungen. Die Pfeile weisen auf die Dorsalbürste. F — aus der Cytopyge austretender Fäkalienballen. Skala 20 µm

ciliatur (Abb. 2, Taf. I 8, 9) gleicht im wesentlichen der Beschreibung von B o h a t i e r et al. (1973). Die Anzahl der Kinetosomen pro Kinete (16–20) ist bei meinem Stamm etwa doppelt so groß. Die Cilien der mittleren Dorsalbürstenreihe weisen die in Abb. 2 (links oben) gezeichnete Form

auf, was mit den Angaben von K a h l (1930–35) übereinstimmt. B o-
h a t i e r et al. (1973) zeichneten dagegen warzenförmige Dorsalborsten,
die K a h l (1930–35) nur bei den zwei seitlichen Reihen feststellte.

Das Silberliniensystem (Abb. 2, Taf. I 8, 9) ist ein sehr engmaschiges
Gitter und gleicht im wesentlichen dem von *Monodinium balbianii* (vgl.
F o i s s n e r 1979 a). Es fehlen aber die horizontal orientierten Maschen
entlang der Kineten. Im Bereich der Dorsalbürste ist das Silberlinien-
system zu größeren Maschen differenziert, infolge der Ausbildung einer
medianen Silberlinie (Abb. 2, Taf. I 8).

(3) Familie *Nassulopsidae* Deroux
Nassulopsis paucivacuolata sp. n. (Abb. 3 a–f, Taf. I 10, 11, II 12–14)

D i a g n o s e: 150–180 µm große, drehrunde, sehr schlanke (etwa 5 : 1)
Nassulopsis mit 3 in einer Reihe angeordneten kontraktilen Vakuolen,
ellipsoiden Makronucleus und blauem, proximalem Pigmentfleck, der aus
vielen, 1–3 µm großen Granula besteht. 40–50 Somakineten, etwa 25 hypo-
stomiale Organellen auf der Ventral- und den Lateralseiten und etwa
10 sehr eng nebeneinander stehende hypostomiale Organellen auf der
Dorsalseite.

L o c u s t y p i c u s: stark eutrophes Kleingewässer (Tümpel 10) auf
der Hochmais-Alm (Großglockner-Hochalpenstraße, etwa 1750 m ü.d.M.).

M o r p h o l o g i e: In der Höhe des Reuseneinganges leicht nach ven-
tral gebogen. Makronucleus stets in Körpermitte und schräg zur Körper-
längsachse liegend. Chromatin netzförmig angeordnet. Reuse proximal
kolbig erweitert, aus etwa 20 leicht tordierten, nach dorsal gerichteten
Stäben bestehend. Pellicula derb, durch die Cilien kräftig gekerbt. Ex-
trusome nicht festgestellt, jedoch liegen dicht unter der Pellicula farblose,
in Reihen angeordnete Granula (Mitochondrien? Extrusome?) (Abb. 3 b).
Erste kontraktile Vakuole dicht unterhalb des Mundeinganges, zweite
etwas unterhalb der Körpermitte, dritte nahe des distalen Poles. Sie
entstehen durch Zusammenfließen vieler kleinerer Vakuolen und ent-
leeren sich ventral über perenne Pori. Entoplasma farblos, wegen der
vielen Nahrungsvakuolen mit Algen (vorwiegend Oscillatorien) in ver-
schiedenen Verdauungsstadien erscheinen die Tiere lebhaft gelb und
grün gefärbt. Auffällig ist die rasche Strömung des Entoplasmas
(Abb. 3 b). Gegen Deckglasdruck sehr empfindlich. Schwimmt mäßig
schnell, kriecht gewandt zwischen Algenfäden und Detritus.

Abstand zwischen zwei Basalkörpern etwa 1.5 µm, zwischen zwei Ki-
neten etwa 2.5 µm (Abb. 3e,f). Oberhalb der hypostomialen Organellen
ist die Anzahl der Kineten der Ventralseite leicht reduziert. Zwischen
den durchlaufenden Kineten sind häufig kurze Kinetensegmente inter-
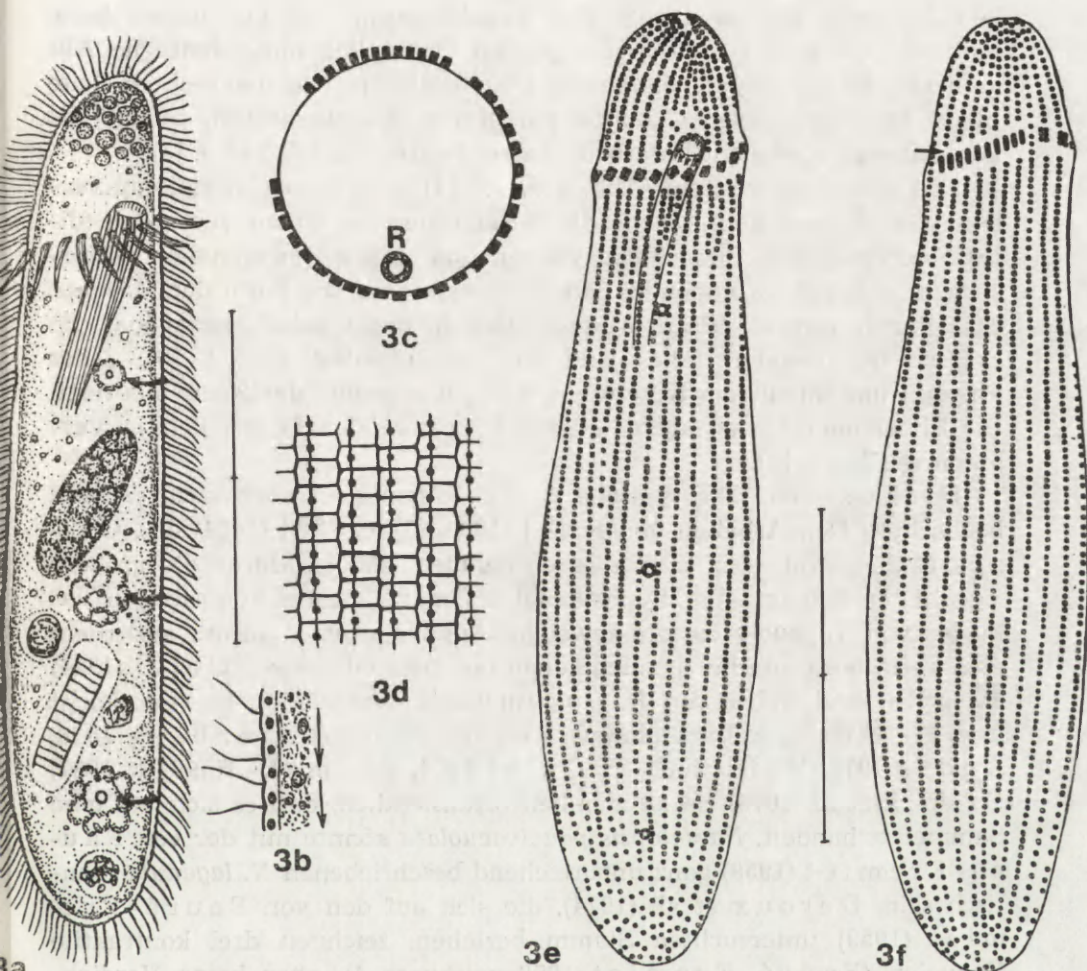
Abb. 3 a–f. *Nassulopsis paucivacuolata*. 3 a — Lateralansicht nach Lebendbeobach-
tungen. Die Pfeile weisen auf die kontraktilen Vakuolen. Skala 70 μm. 3 b — Teil
der Pellicula. Die Pfeile symbolisieren das rasch strömende Entoplasma. 3 c —
Schematisierter Querschnitt in der Höhe der hypostomialen Organellen. R — Reuse.
3 d — Teil des Silberliniensystems nach trockener Silberimprägnation. 3 e — In-
fraciliatur der Ventralseite nach nasser Silberimprägnation. Skala 55 μm. 3 f —
Dasselbe Individuum wie in Abb. 3 e, aber durchfokussiert, um die Infraciliatur
der Dorsalseite zu zeigen. Skala 55 μm.

caliert, besonders dicht unterhalb der hypostomialen Organellen. Im Be-
reich des distalen Poles sind die Basalkörper lockerer angeordnet. Pro-
ximal konvergieren die Kineten entlang einer sehr kurzen Nahtlinie
(vgl. D e r o u x et al. 1974).

Unterhalb des Reuseneinganges verläuft leicht schräg das hyposto-
miale Organellenband (Abb. 3a,e,f, Taf. II 12, 14). Die Organellen beste-

hen aus zwei Reihen mit je drei Basalkörpern. Auf der linken Seite schließen die weit gestellten marginalen Organellen ohne deutliche Abgrenzung an die eng stehenden der Dorsalseite an. Auf der rechten Seite bleibt zwischen diesen Gruppen ein breiter Zwischenraum, so daß die Somakineten hier nicht unterbrochen sind (Abb. 3 c,e,f, Taf. I 11).

Das Silberliniensystem (Abb. 3 d, 10, 11) besteht aus meridional verlaufenden Silberlinien, welche die Basalkörper der Cilien verbinden (direkt verbindendes Silberliniensystem) und einem polygonalen Gittersystem (indirekt verbindendes Silberliniensystem). Die Form der Maschen ist ziemlich variabel (Präparationsartefakt?), meist jedoch hexagonal. Im Bereich des distalen Poles sind sie unregelmäßig (Taf. I 10). Jedes Polygon umschließt ein Kinetosom, das etwas rechts des Zentrums liegt. Die Silberlinien treten auch mit den hypostomialen Organellen in Verbindung (Taf. I 11).

Diskussion: Die typische Art, *N. elegans,* ist noch unzureichend beobachtet. Den Arbeiten von K a h l (1930–35), G e l e i (1954) und C z a p i k et al. (1976) kann man aber entnehmen, daß sie durch die größere Anzahl von Somakineten, hypostomialen Organellen und kontraktilen Vakuolen von *N. paucivacuolata* abweicht. Aus Wegpfützen ist mir außerdem eine Form bekannt, die ziemlich genau der Darstellung von G e l e i (1954) entspricht und sicher von *N. paucivacuolata* verschieden ist. Vergleicht man die bisherigen Darstellungen von *N. elegans* (E h r e n b e r g 1838, A n d r é 1916, K a h l 1930–35, G e l e i 1954, Š r á m e k - H u š e k 1957, C z a p i k et al. 1976), so ist ziemlich offensichtlich, daß es sich um eine Sammelart handelt. *Nassulopsis paucivacuolata* könnte mit der von F a u r é - F r e m i e t (1959) ganz unzureichend beschriebenen *N. lagenula* identisch sein. D e r o u x et al. (1974), die sich auf den von F a u r é - F r e m i e t (1959) untersuchten Stamm beziehen, zeichnen drei kontraktile Vakuolen (F a u r é - F r e m i e t 1959 zeichnete 4), aber keine Verdichtung der hypostomialen Organellen auf der Dorsalseite. Ich nehme jedoch an, daß diese ein Genuskriterium ist, da ich sie auch bei der Form aus Wegpfützen festgestellt habe. T u c k e r (1971) stellte bei einer typischen (?) *Nassula* ebenfalls ein verdichtetes Feld dorsaler hypostomialer Organellen fest.

Systematische Stellung: Das Genus *Nassulopsis* wird von C o r l i s s (1977) in die Ordnung *Synhymeniida* gestellt. Dieser Einordnung entspricht das hypostomiale Organellenband. Das Silberliniensystem gleicht dagegen dem gewisser *Nassula*-Arten (F o i s s n e r, unveröffentlicht). Daher erscheint mir diese Einordnung zweifelhaft, zumal ein typischer Vertreter der *Synhymeniida, Chilodontopsis depressa,* ein ganz anderes Silberliniensystem besitzt (s. unten). P u y t o r a c et al. (1974) trennten *Nassulopsis* als eigene Unterordnung von den *Synhymenina* ab.

Das scheint mir eine günstige Möglichkeit zu sein, dem unterschiedlichen Silberliniensystem Rechnung zu tragen, wenn man *Nassulopsis* nicht so wie J a n k o w s k i (1968) zu den *Nassulina* stellen will.

(4) Familie *Orthodonellidae* Jankowski
Chilodontopsis depressa (Perty, 1852) (Abb. 4 a–d, Taf. II 15–17)

M o r p h o l o g i e: Der 60–70 µm große Ciliat entspricht im wesentlichen den Beschreibungen von B l o c h m a n n (1895) und K a h l (1930–35). Im Umriß fast rechteckige Individuen (B l o c h m a n n 1895, R o u x 1901, K a h l 1930–35) beobachtete ich nicht. Durch das nach links oben vorgebogene Vorderende (vgl. K a h l 1930–35) sind die Tiere bei schräger Ansicht leicht S-förmig gekrümmt (Abb. 4 c). Dies verursacht vermutlich die etwas unbeholfen erscheinende Bewegung. Cilien lang und weich. Außerdem einige gering verlängerte, ziemlich starre "Tastborsten". Makronucleus ellipsoid, zentral gelegen, mit einer tiefen Einkerbung, in der ein ellipsoider Mikronucleus liegt. Reuse nach links und dorsal gerichtet, aus 12–14 Stäben aufgebaut. Um ihre Mündung zieht eine weiche Membran. Kontraktile Vakuole auffällig groß (vgl. K a h l 1930–35), entleert sich in langen Abständen terminal über einen perennen Porus. Sie entsteht durch Zusammenfließen von kleineren Vakuolen. Entoplasma hyalin, mit wenigen, meist leicht gelblichen Granula und Nahrungsvakuolen mit Grünalgen und Bakterien. Kriecht meist auf Pflanzenresten und Detritushäufchen.

20–25 ventrale und ca. 10 dorsale Kineten (Abb. 4 b,d, Taf. II 15,16). Etwa 13 ventrale Wimperreihen stoßen postoral an die hypostomialen Organellen, die restlichen ziehen rechts an der Reuse vorbei, biegen nach links um und stoßen vor dem Mund an die hypostomialen Organellen. 2–3 kurze Kineten stoßen links an die Reuse. Im distalen Drittel stehen die Cilien lockerer, besonders auf der Dorsalseite, die überhaupt spärlicher bewimpert ist (Abb. 4 d). Die Basalkörper erscheinen aus zwei argyrophilen Körnchen zusammengesetzt. Das hypostomiale Organellenband beginnt auf der linken Lateralseite und verläuft von links oben nach rechts unten. Es endet etwas rechts der Reuse und besteht aus 20–25 cirrenartigen Organellen, die aus je drei Basalkörpern aufgebaut sind (Abb. 4 b, Taf. II 15,16). Das Silberliniensystem besteht aus wellig verbogenen, meridional verlaufenden Silberlinien, welche die Basalkörper verbinden und ebenfalls wellig verbogenen, horizontal orientierten Silberlinien, welche die Kineten untereinander verbinden. Vereinzelt liegen argyrophile Körnchen in den Silberlinien. Es sind vielleicht die Basalkörper der Tastborsten oder Relationskörper von Extrusomen.
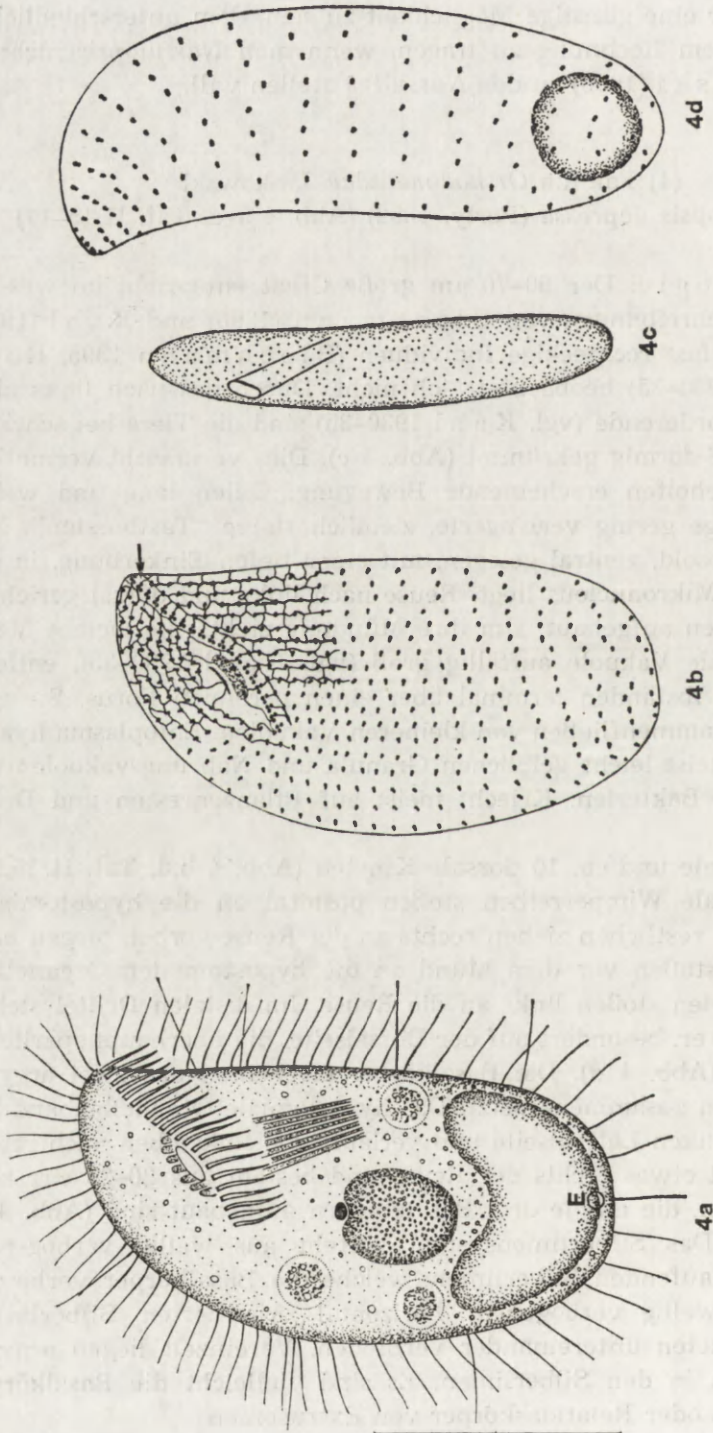
Abb. 4 a-d. *Chilodontopsis depressa*. 4 a — Ventralansicht nach Lebendbeobachtungen. E — Exkretionsporus der kontraktilen Vakuole. Skala 30 μm. 4 b — Infraciliatur und Silberliniensystem (nur rechts oben eingezeichnet) der Ventralseite nach trockener Silberimprägnation. Der Pfeil weist auf das hypostomiale Organellenband. 4 c — Lateralansicht nach Lebendbeobachtungen. 4 d — Infraciliatur der Dorsalseite nach trockener Silberimprägnation

Systematische Stellung: Die systematische Stellung des Genus *Chilodontopsis* ist unklar (Jankowski 1968). Deroux (in Corliss 1977) stellt es in die Familie *Scaphidiodontidae*. Ich glaube aber, daß man *Chilodontopsis* auch in die Familie *Orthodonellidae* Jankowski, 1968 einreihen kann, da die Infraciliatur ganz ähnlich wie die der Genera *Orthodonella* und *Synhymenia* ist (s. Jankowski 1968). *Chilodontopsis vorax* ist nicht congenerisch mit *C. depressa*, da die Infraciliatur (s. Agamaliev 1967) der des Genus *Synhymenia* entspricht.

Das Silberliniensystem der meisten in der Ordnung *Synhymeniida* zusammengefaßten Ciliaten (Corliss 1977) ist leider nicht bekannt. Jenes von *Chilodontopsis depressa* repräsentiert einen bisher bei Ciliaten nicht beobachteten Typus. Das weist auf eine Sonderstellung dieser Gruppe hin.

DANKSAGUNG

SUMMARY

The morphology, infraciliature, and silverline system of some ciliates (*Dileptus anser, Dileptus visscheri, Acropisthium mutabile, Nassulopsis paucivacuolata* nov. spec., *Chilodontopsis depressa*) occurring in the Großglockner area (Hohe Tauern, Austria) is described. The systematic status of the genera *Nassulopsis* and *Chilodontopsis* is briefly discussed. *Nassulopsis* seems to be related to the *Synhymeniida* and *Nassulida*. *Chilodontopsis* is a typical synhymenal ciliate with a particularly differentiated silverline system.

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LEGENDEN ZU DEN TAFELN I–II

5,7: *Dileptus anser.* Teile der Infraciliatur und des Silberliniensystems des mittleren Körperabschnittes nach nasser und nach trockener Silberimprägnation. Die Pfeile weisen auf die Somakineten

6: *Dileptus anser.* Infraciliatur und Silberliniensystem im distalen Polbereich. Trokkene Silberimprägnation

8,9: *Acropisthium mutabile.* Infraciliatur und Silberliniensystem konjugierender Individuen nach trockener Silberimprägnation. Das Silberliniensystem ist im Bereich der Dorsalbürste (Db) zu größeren Maschen differenziert. Die Pfeile weisen auf den Pektinellenkranz

10,11: *Nassulopsis paucivacuolata.* Infraciliatur und Silberliniensystem des distalen Poles und der Dorsalseite des proximalen Körperabschnittes nach trockener Silberimprägnation. Die Pfeile weisen auf das jeweils letzte weit gestellte hypostomiale Organell der Lateralseiten. Der Doppelpfeil weist auf die eng gestellten hypostomialen Organellen der Dorsalseite

12,13,14: *Nassulopsis paucivacuolata* nach nasser Silberimprägnation. Abb. 12 zeigt die Infraciliatur der Ventralseite, Abb. 14 ein stärker vergrößertes Detail davon. Die vollen Pfeile weisen auf die Exkretionspori der kontraktilen Vakuolen bzw. in Abb. 13 auf den proximalen Pigmentfleck. Die offenen Pfeile weisen auf die hypostomialen Organellen

15,16,17: *Chilodontopsis depressa.* Infraciliatur und Silberliniensystem der Ventralseite nach trockener Silberimprägnation. Abb. 17 zeigt einen stärker vergrößerten Ausschnitt von Abb. 15. Der Pfeil in Abb. 16 weist auf das hypostomiale Organellenband
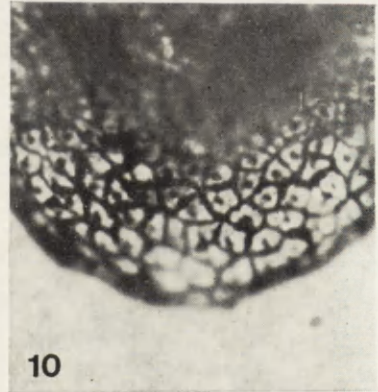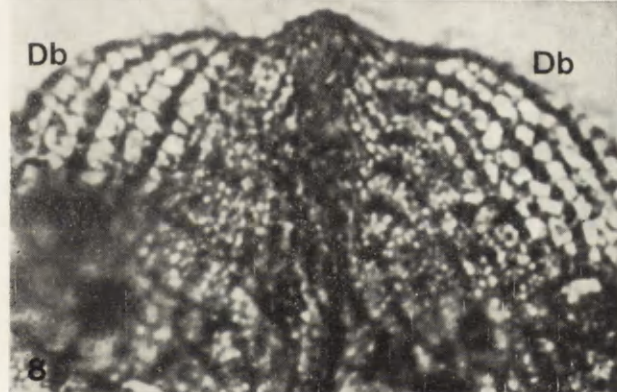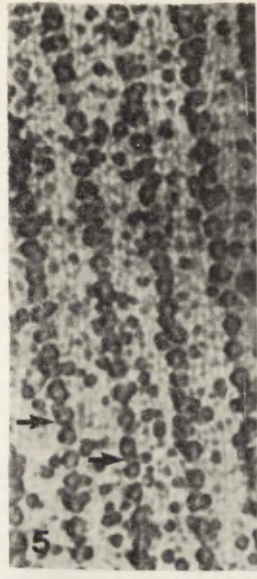
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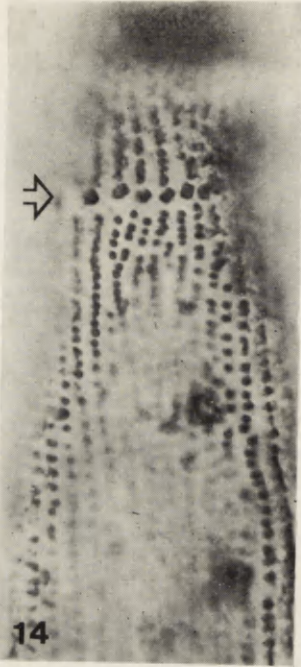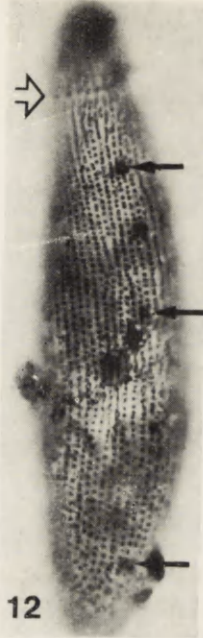
V a s s i l GOLEMANSKY

Cyphoderia compressa sp. n. (*Rhizopoda: Arcellinida*) — un nouveau thécamoebien psammobionte de supralittoral des mers

Synopsis. On donne la description d'un taxon nouveau *Cyphoderia compressa* sp. n. La nouvelle espèce a été trouvée dans les eaux souterraines des plages maritimes sabloneuses et elle est considérée comme un psammobionte.

Du genre *Cyphoderia* Schlumberger, 1845 sont décrites jusqu'à présent 17 espèces et varietées, habitant surtout des biotopes dulçaquicoles: lacs, rivières, mers, sphaignes, mousses humides et submergées, différents types du sol. La première espèce trouvée dans des biotopes marins c'est *C. ampulla* (Ehrenberg, 1840) Schlumberger, 1845, considerée aussi comme une ubiquiste (Z e r n o v 1949). Elle est trouvée pour la première fois dans la Mer Baltique par S c h u l z e (1874) et plus tard retrouvée dans différents biotopes marins et saumâtres par L e v a n d e r (1894), F l o r e n t i n (1899, cité par R u i n e n and B e c k i n g 1938), M ö b i u s (1899), W a i l e s (1927), G u r v i t s c h (1934), H o o g e n r a a d and D e G r o o t (1940), R e m a n e (1950), B i e r n a c k a (1962, 1963, 1967), G o l e m a n s k y (1970, 1971), C h a r d e z (1977) etc.

S c h u l z e (1874) signale d'avoir trouvé dans un aquarium avec de l'eau de la Mer Baltique une *Cyphoderia* dont l'axe longuitudinale est une ligne droite et le pseudostome circulaire est disposé en angle droit sur cet axe. Mais P e n a r d (1902) indique qu'il est "peu probable qu'il existe une *Cyphoderia* déporvue de toute courbure..." En réalité cette espèce de Schulze n'est pas retrouvée depuis sa première description.

En 1973, au cours d'une étude sur les thécamoebiens du psammal supralittoral de la Mer Baltique nous avons décrit une nouvelle espèce du genre *Cyphoderia*: *C. littoralis* Gol., 1973, considerée par nous comme un psammobionte strict des mers (G o l e m a n s k y 1973, 1974, 1976). Nos recherches ultérieures sur les thécamoebiens psammobiontes des

mers nous ont montré que les eaux souterraines des plages sableuses marines sont souvent habitées et par une troixième espèce du genre *Cyphoderia*, dont la description complète est présentée ci-dessous.

Cyphoderia compressa sp. n. Fig. 1 a, b.; Planche I 1–5

D e s c r i p t i o n: Théque allongée, colorée jaune-pâle et transparente. Vue de profil elle a la forme typique d'une *Cyphoderia*: le fond arrondie et la partie antérieure rétrecie et récourbée en forme de retorte
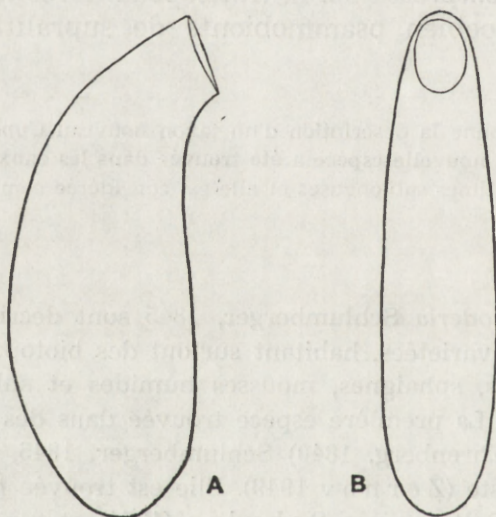
Fig. 1. *Cyphoderia compressa* sp. n., a — vue de profil, b — vue sagitale

(Fig. 1 a, Pl. I, 1,3). Rarement on observe des thèques dont le fond est plus ou moins coupé droit (Pl. I 2). De vue ventrale la thèque est nettement comprimée sur toute la longueur (Fig. 1 b). La section transversale et le pseudostome sont ovales ou élliptiques.

Le revêtement de la thèque est semblable à ce de *C. ampulla*. D'ailleurs il nous semble utile de rappeler ici que le revêtement de *C. ampulla* est très varié. D'après L e i d y (1879) la structure de la thèque est chitinoïde et "composed of minute hexagonal elements of uniform size arranged in alternating series in parallel spiral rows". P e n a r d (1902) écrit que la surface de la thèque du genre *Cyphoderia* est "tout entière guillochée de dessins extréméments petits, mais bien nets, hexagonaux, régulier, donnant l'apparence de séries alternantes de rangées spirales qui se coupent les unes les autres sous des angles aigus ou obtus suivant la manière dont on les considère". En caractérisant l'espèce *C. ampulla*,

Penard précise que la thèque est "composée tout entière de petits disques rondes se touchant par leurs bords sans imbrication... Ces disques sont disposés en un ordre très régulier, et présentent alors dans leur ensemble l'apparence d'alvéoles hexagonaux qui forment par leur réunion symmétrique des systèmes de lignes droites s'entrecoupant sous différents angles". Dans le cas de *C. compressa* sp. n. le revêtement est formé aussi de petits disques ovales, collés l'un près de l'autre sur la base chitinoide de la thèque (Pl. I 4). Parfois on observe des disques dont la forme n'est pas tout à fait ronde, mais ils ont toujours des bords arrondis (Pl. I 5).

Les dimensions observées des thèques sont:

> Longueur: 82–112 μm;
> Largeur: 30–46 μm;
> Epaisseur: 20–30 μm;
> Pseudostome: 10–18 × 7–12 μm.

Dans les eaux souterraines littorales des plages marines on trouve souvent des thèques vides et des exemplaires vivants de la nouvelle espèce. Ordinairement les animaux vivants émettent plusieurs filopodes. Au microscope photonique le corps cytoplasmique ne diffère pas du ce de *C. ampulla*. Le noyau est assez volumineux et atteint 23 μm de diamètre. Très souvent autour de lui on observe des inclusions cytoplasmiques (petits corps noires de forme arrondie), décrites pour la première fois par P e n a r d (1902) chez *Cyphoderia* et *Campascus* du lac Léman. Ces inclusions cytoplasmiques sont observées aussi ches les animaux inkystés et cela nous donne la raison de supposer qu'il s'agit des reserves nutritives, utilisées par les animaux au cours de leur diapause ou de leur réproduction (Pl. I 2).

E c o l o g i e: *C. compressa* sp. n. habite les eaux souterraines littorales des plages sableuses des mers. Elle est une espèce euryhaline, survivant les variations de la salinité de 1.33% (Mer Baltique) jusqu'au 16.98% (Mer Noire). En ce qui concerne la profondeur dans le sable l'espèce a été trouvée à un maximum de 1.20 m. *C. compressa* sp. n. a été souvent observée en populations de *C. ampulla* et *C. littoralis*. Elle vie assez longtemps et dans les cultures au laboratoire où on observe souvent des individus en état de division (Pl. I 3).

R é p a r t i t i o n g é o g r a p h i q u e: *C. compressa* sp. n. a été trouvée jusqu'à présent dans plusieurs stations sur les plages sableuses de la Mer Noire en Bulgarie, la Mer Baltique en Pologne et une fois seulement dans les eaux souterraines littorales de Nanaimo (Canada, Pacifique).

Matériel: Une préparation "Holotype" et une autre — "Paratype" sont gardées dans la collection de l'auteur à l'Institut de Zoologie de Sofia. Elles proviennent de la Mer Noire.

Discussion: *C. compressa* sp. n. se distingue de *C. littoralis* Gol. par la forme générale de la thèque, qui n'est jamais lancéolées au fond comme le cas de la dernière espèce et par ces dimensions présque deux fois plus grandes. De plus, nous n'avons jamais observé une collerette chitinoide autour de pseudostome de *C. compressa* sp. n., tandis qu'une telle collerette est très souvent chez *C. littoralis*.

Il est plus difficile de distinguer *C. compressa* sp. n. de l'espèce *C. ampulla* (Ehrenberg) Schlumberger qui habite les mêmes biotopes. En vue de profil c'est la largeur relativement plus grande de la thèque qui indique la présence de la nouvelle espèce. Mais de vue sagitale on remarque facilement que la thèque de *C. compressa* sp. n. est fortement comprimée et le pseudostome est toujours ovale ou élliptique. Rappelons que le pseudostome de *C. ampulle* est "toujours ronde, à peu près ventrale, et coupe le col en biais" (Penard 1902).

REMERCIEMENT

SUMMARY

The description of a new taxon *Cyphoderia compressa* sp. n. is given. The new species is found in the groundwater of the marine sandy beaches and is considered as a psammobionte.

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EXPLICATION DE PLANCHE I

Cyphoderia compressa sp. n.

1: exemplaire vivant
2: exemplaire inkysté
3: division
4: revêtement de la théque
5: détail du revêtement de la théque

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Cephaline Gregarine *Leidyana linguata* sp. n. Parasite of a Gryllid *Pteronemobius concolor* Walker from India

Synopsis. A new species of cephaline gregarine (*Protozoa : Sporozoa*), *Leidyana linguata* is described from the mid gut of a gryllid, *Pteronemobius concolor* Walker. The gregarine has a ratio of LP : TL = 1 : 5.5 and WP : WD = 1 : 1.3. The genus is reported for the first time from an Indian insect.

Watson (1915), in her work on the polycystid gregarines, created a new genus *Leidyana* and transferred some previously described species by Cuenot (1901) and Crawley (1907) to this genus. The genus *Leidyana* was defined as having solitary sporadins, epimerite simple, globular, sessile knob and cyst with duct with dolioform spores. Later, Keilin (1918) described a new species, *L. tinei* from a lepidopteran larva and Daviault (1929) reported *L. ephestiae* from *Ephestia kühniella* Zell. Recently, Hoshide (1958), Théodoridès and Echard (1962), Baudoin (1966), Geus (1966) Ormières (1966) and Corbell (1968) have contributed in the understanding of the genus *Leidyana* Watson and also described many new species under this genus.

In course of our studies on the cephaline gregarines (*Protozoa : Sporozoa*) from insects, we obtained a form from the mid gut of a gryllid, *Pteronemobius concolor* Walker having a simple sessile, globular epimerite and cyst with sporoduct but never with sporadins in syzygy (caudo-frontal association) and as such could be placed under the genus *Leidyana* Watson, 1915. The parasite is designated here as a new taxon and its structure and life cycle are also described in details. As far as we are aware, this is the first report of a *Leidyana* species from an Indian insect.

Material and Methods

The insects were brought alive to the laboratory and their alimentary canals were dissected out in 0.5 per cent saline solution. Smears of infected mid gut contents were made on grease-free slides and fixed in Schaudinn's fixative. The highly infected mid guts were fixed in Bouin's fluid and 5.0 μm thick serial sections were made to study the intracellular development of the parasite. Both smears and sections were stained subsequently with iron alum-haematoxylin method. The development of the gametocysts was observed after placing them in cavity slide with a drop of 0.5 per cent saline solution and keeping them in moist chambers. The spores were examined with Lugol's iodine solution at regular intervals of six hours.

The figures have been drawn with the help of a camera lucida. The ratios used in this paper are the ratio of length of protomerite to total length and the ratio of width of protomerite to width of deutomerite.

Observations

Structure of the Trophozoite and Sporadin

The earliest stage of the gregarine obtained from mid gut smear is a trophozoite (Fig. 1 1), having epimerite, protomerite and deutomerite. The epimerite is large and characteristically tongue-like being broadest near its base. It measures 15.7 × 7.0 μm. The protomerite is dome-shaped whereas the deutomerite is subspherical containing a spherical nucleus towards its anterior end. Its posterior end is round. The septum between epimerite and protomerite is not so distinct as it is between protomerite and deutomerite. The trophozoite measures 53.4 × 17.3 μm in the average.

The sporadins (Fig. 1 2 and 3) are elongated bodies and, as usual, consist of protomerite and deutomerite. These measure 31.4 to 415.8 μm in length and 12.5 to 71.8 μm in width. In smaller forms (Fig. 1 2), the protomerite is dome-shaped or hemispherical but in larger forms (Fig. 1 3), this is conical measuring 9.4 to 77.0 μm in length and 9.4 to 49.0 μm in width. A part of the epimerite remains attached with the anterior end of the protomerite (Fig. 1 2). The deutomerite is elongated and cylindrical measuring 22.0 to 338.0 μm in length and 12.5 to 71.8 μm in width. In smaller sporadins, this is broadest slightly below the septum and then suddenly narrows down to half of its width whence its margins run parallel towards the posterior side and end bluntly. In larger forms, however, the deutomerite is broadest near the posterior two-third position and then tapers gradually in a pointed proximity.

In trophozoites and smaller form of sporadins the cytoplasm is uniform throughout, consisting of fine granules (Fig. 1 1 and 2), whereas
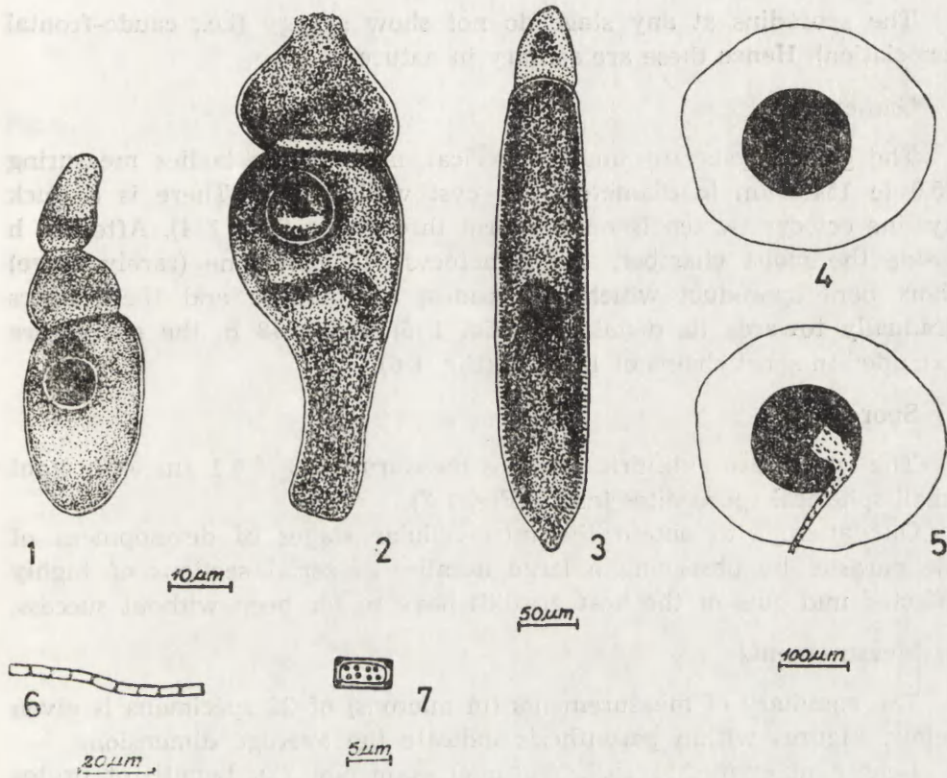
Fig. 1. *1* to *7* — Camera Lucida drawings of *Leidyana linguata* sp. n. *1* — Tropho-zoite with characteristic tongue-like epimerite, *2* — Smaller form of sporadin with ovoid nucleus and a transverse slit-like hyaline space just below the karyosome, *3* — Larger form of sporadin where posterior edge of the nucleus is tethered with myoneme fibrils, *4* — Cyst with thick hyaline ectocyst, *5* — Cyst with a short, bent sporoduct, *6* — Spores in chain, *7* — A mature spore with sporozoites inside

in larger forms of sporadins, the protomerite is finely granulated and the deutomerite is filled up with fine and coarse granules. In all the forms the cytoplasm is differentiated into an outer hyaline epicyte, an inner granular endocyte and longitudinal rows of myonemes in between.

The nucleus is round in trophozoites and round to almost ovoid in smaller sporadins consisting of a nuclear membrane and a centrally placed round karyosome having a hyaline space just below it (Fig. 1 2). In larger sporadins, the nucleus is almost in the middle of the deuto-merite and stains deeply with Heidenhain's haematoxylin. Its shape is highly variable exhibiting hemispherical semilunar, spindle-shaped or oval forms. The nucleus is tethered posteriorly by many fine, thread-like structures which R a y and C h a k r a v a r t y (1933) supposed as myoneme fibrils. The nucleus measures 6.3 to 11.0 µm in diameter.

The sporadins at any stage do not show syzygy (i.e., caudo-frontal association). Hence these are solitary in nature.

Gametocyst

The gametocysts are small, spherical, milky-white bodies measuring 75.0 to 154.0 μm in diameter. The cyst wall is thin. There is a thick hyaline ectocyst which is not uniform throughout (Fig. 1 4). After 24 h inside the moist chamber, the gametocyst develops one (rarely three) short bent sporoduct which is broadest at its base and then tapers gradually towards its distal end (Fig. 1 5). After 48 h, the spores are extruded in short chains of 15 to 20 (Fig. 1 6).

Spore

The spores are cylindrical bodies measuring 9.8 × 4.1 μm with eight small spherical sporozoites inside (Fig. 1 7).

Our attempt to obtain the intra-cellular stages of development of the parasite by observing a large number of serial sections of highly infected mid guts of the host gryllids have so far been without success.

Measurements

The summary of measurements (in microns) of 22 specimens is given below. Figures within parenthesis indicate the average dimensions.

Length of epimerite 15.7, Width of epimerite 7.0, Length of proto-merite 9.4–77.0 (25.9), Width of protomerite 9.4–49.0 (20.7), Length of deutomerite 22.0–338.8 (117.6), Width of deutomerite 12.5–71.8 (27.5), Total length 31.4–415.8 (143.5), Total width 12.5 to 71.8 (27.5), Diameter of the nucleus 6.3 to 11.0 (9.1).

LP : TL = 1 : 5.5, WP : WD = 1 : 1.3

The details of measurements of these specimens are shown in Table 1.

Seasonal Intensity and Site of Infestation

The infection is maximum durin the months of September and October. During other months, very scanty infection is noted. On an average 10.1 per cent of the insects are parasitized. The seat of infec-tion is the mid gut.

Material

Holotype on slide No. 06/3 obtained from smears of mid gut of the gryllid, *Pteronemobius concolor* Walker, collected by N. K. Sarkar from Kalyani, West Bengal, India on May 8, 1977. Paratypes, many, on the above numbered slide and on other slides, other particulars are the same as for the holotype material.

Table 1

Measurements (in microns) of different parts of 22 specimens of *Leidyana linguata* sp. n.

Number	TL	LE	LP	LD	WE	WP	WD	N	LP:TL	WP:WD
1	53.4	15.7	11.0	26.7	7.0	12.5	17.3	6.3	1:3.4	1:1.3
2	196.0	—	28.0	168.0	—	24.5	42.0	—	1:7.0	1:1.7
3	168.0	—	28.0	140.0	—	21.0	24.5	—	1:6.0	1:1.1
4	168.0	—	28.0	140.0	—	21.0	17.5	—	1:6.0	1:0.8
5	154.0	—	28.0	126.0	—	21.0	28.0	—	1:5.5	1:1.3
6	168.0	—	28.0	140.0	—	21.0	28.0	—	1:6.0	1:1.3
7	182.0	—	28.0	154.0	—	28.0	45.5	—	1:6.5	1:1.6
8	196.0	—	28.0	168.0	—	28.0	31.5	—	1:7.0	1:1.1
9	98.0	—	14.0	84.0	—	14.0	21.0	—	1:7.0	1:1.5
10	224.0	—	42.0	182.0	—	21.0	38.5	—	1:5.3	1:1.8
11	415.8	—	77.0	338.8	—	49.0	71.8	—	1:5.4	1:1.4
12	140.0	—	28.0	112.0	—	21.0	21.0	—	1:5.0	1:1.0
13	210.0	—	42.0	168.0	—	21.0	28.0	—	1:5.0	1:1.3
14	126.0	—	14.0	112.0	—	17.5	21.0	—	1:9.0	1:1.2
15	168.0	—	28.0	140.0	—	17.5	31.5	—	1:6.0	1:1.8
16	80.0	—	18.0	61.2	—	25.1	28.3	11.0	1:4.2	1:1.1
17	72.2	—	18.8	53.4	—	15.7	17.3	9.4	1:3.8	1:1.1
18	83.2	—	22.0	61.2	—	18.8	22.0	9.4	1:3.7	1:1.1
19	56.5	—	9.4	47.1	—	14.1	17.3	—	1:6.0	1:1.2
20	78.5	—	18.8	59.7	—	18.8	19.6	9.4	1:4.1	1:1.0
21	31.4	—	9.4	22.0	—	9.4	12.5	—	1:3.3	1:1.3
22	88.0	—	22.0	66.0	—	15.7	18.8	—	1:4.0	1:1.1

Abbreviations: TL — total length, LE — length of epimerite, LP — length of protomerite, LD — length of deutomerite, WE — width of epimerite, WP — width of protomerite, WD — width of deutomerite, N — diameter of the nucleus.

Slides bearing the holotype and paratype materials are deposited presently to the Department of Zoology, University of Kalyani, Kalyani, West Bengal, India, to be sent finally to the National Collection of the Zoological Survey of India, Calcutta.

Discussion

In having solitary sporadins, simple, sessile epimerite, cysts with ducts and cylindrical spores, the gregarine described herein undoubtedly belongs to the genus *Leidyana* Watson, 1915. However, in determining its true generic status, utmost care ought to be taken because the characters of the genus *Leidyana* Watson are also shared by the genus *Gregarina* Dufour, except that in the latter the sporadins are always biassociative. The gregarine differs from the previously described ones under *Leidyana*

in measurements, characteristic tongue-like epimerite, nucleus tethered with myoneme fibrils in larger sporadins and host range. It is, therefore, considered to be a new taxon for which the name *Leidyana linguata* new species is proposed. The specific trivial name has been given to stress the characteristic tongue-like epimerite, and has been derived from the Latin word "lingua" meaning tongue.

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RÉSUMÉ

    Le travail contient les descriptions de la morphologie et du cycle de développment d'une nouvelle espèce des grégarines (*Protozoa : Sporozoa*), *Leidyana linguata* (LP : TL = 1 : 5.5; WP : WD = 1 : 1.3) de l'intestin de l'gryllides, *Pteronemobius concolor* Walker aux Indes.

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Two New Septate Gregarines, *Dendrorhynchus keilini* sp. n. and *Ancyrophora ceriagrioni* sp. n. from the Midgut of the Damsel Fly, *Ceriagrion coromandelianum* (Fabr.) [2]

Synopsis. The morphology and life-history of two new species of septate gregarines, *Dendrorhynchus keilini* sp. n. and *Ancyrophora ceriagrioni* sp. n. from the midgut of the damsel fly *Ceriagrion coromandelianum* (Fabr.) is described. In *Dendrorhynchus keilini* the trophozoites reach a maximum size of 400×300 µm. The epimerite is expanded into a disc-like structure, the margin of which is produced into 13–16 bifid papillae which partly penetrate the epithelial cell. Cysts reach a diameter of 255 µm and have an ectocyst 30 µm thick. Gametes isogamous measuring 7.5×4.5 µm. Dehiscence simple, spores oval measuring 7.0×3.0 µm. Cystal and sporocyst residuum present. The trophozoites of *Ancyrophora ceriagrioni* measure 900×200 µm and the sporonts measure 825×150 µm. Epimerite is in the form of a shallow bowl, the margin of which is produced into 13–16 digitiform processes. Cysts spherical having a diameter of 255–350 µm with an ectocyst 40 µm thick. Sporogony completed in 72 h. Spores biconical measuring $6.5 \times \times 5.4$. Four polar and four equatorial spines present.

While examining the gut contents of the damsel fly, *Ceriagrion coromandelianum* (Fabr.) collected from different localities in Visakhapatnam, Andhra Pradesh (India) we came across two septate gregarines belonging to the genera, *Dendrorhynchus* Keilin, 1920 and *Ancyrophora* Leger, 1892. Since K e i l i n (1920) described *Dendrorhynchus systeni* from the gut of the larvae of the dolichopodid fly, *Systenus* sp. (Probably *Systenus scholtzii* Louw) there has so far been no report of any other species belonging to the genus *Dendrorhynchus* and the present form does not resemble the only other described species in all its features and hence

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is considered new and the name *Dendrorhynchus keilini* sp. n. is proposed for the same. The second gregarine is also considered new because it does not resemble any other species of *Ancyrophora* described so far in all its features and hence is considered new and the name *Ancyrophora ceriagrioni* sp. n. is proposed for the same.

Material and Methods

The damsel flies, *Ceriagrion coromandelianum* (Fabr.) were collected from two different localities during May–July 1975. The insects which harboured *Dendrorhynchus keilini* sp. n. were collected from the dairy farm area in Visakhapatnam and those which harboured *Ancyrophora ceriagrioni* from around a pond of fresh water in the Shanthi Ashram area in Waltair Uplands. In both cases the insects were caught on the wing using an insect trapping net. Soon after they were brought to the laboratory they were isolated and kept in glass finger-bowls covered with net at the bottom of which a moist blotting paper was placed to keep the faecal matter passed out in a moist condition. No attempt was made to feed the flies and they remained active and alive for about 2–3 days.

All insects collected were decapitated and the gut contents were examined for the parasites which could be seen through the transluscent gut wall. Observations on the unfixed parasites were made by teasing out a bit of the infected portion of the gut on a slide and examining them in the body fluid of the host, supplemented with a drop of Ringer's solution when necessary.

Smears were wet-fixed in Schaudinn's fluid and stained with Ehrlich's acid haematoxylin. Material for sectioning was fixed in alcoholic Bouin's fluid, sectioned at 8 μm thickness and stained with Heidenhain's iron haematoxylin. Cysts collected from the hind gut of the host and those collected from the faecal matter were kept in 2.5% aqueous Potassium dichromate and examined at intervals to observe gametogenesis and sporogony. Smears showing gametes were fixed in Schaudinn's fluid and stained with Ehrlich's acid haematoxylin while those showing the spores were fixed in Carnoy's fluid and treated according to Feulgen's technique because other methods of staining the spores were unsuccessful.

Observations

Dendrorhynchus keilini sp. n.

Diagnosis. Cephalonts 200–400 μm × 70–130 μm, epimerite with short neck, expanded distally into a disc-like structure the margin of which is produced into 13–16 bifid papillae; sporonts 400 × 150 μm, cysts 255 μm in diameter having an ectocyst 30 μm thick; Gametes isogamous, oval measuring 7.5 × 4.5 μm; Dehiscence simple, spores oval 12.0 × 5.0 μm, Octozoic.

60 out of 180 (33%) of adult *Ceriagrion coromandelianum* (Fabr.) col-
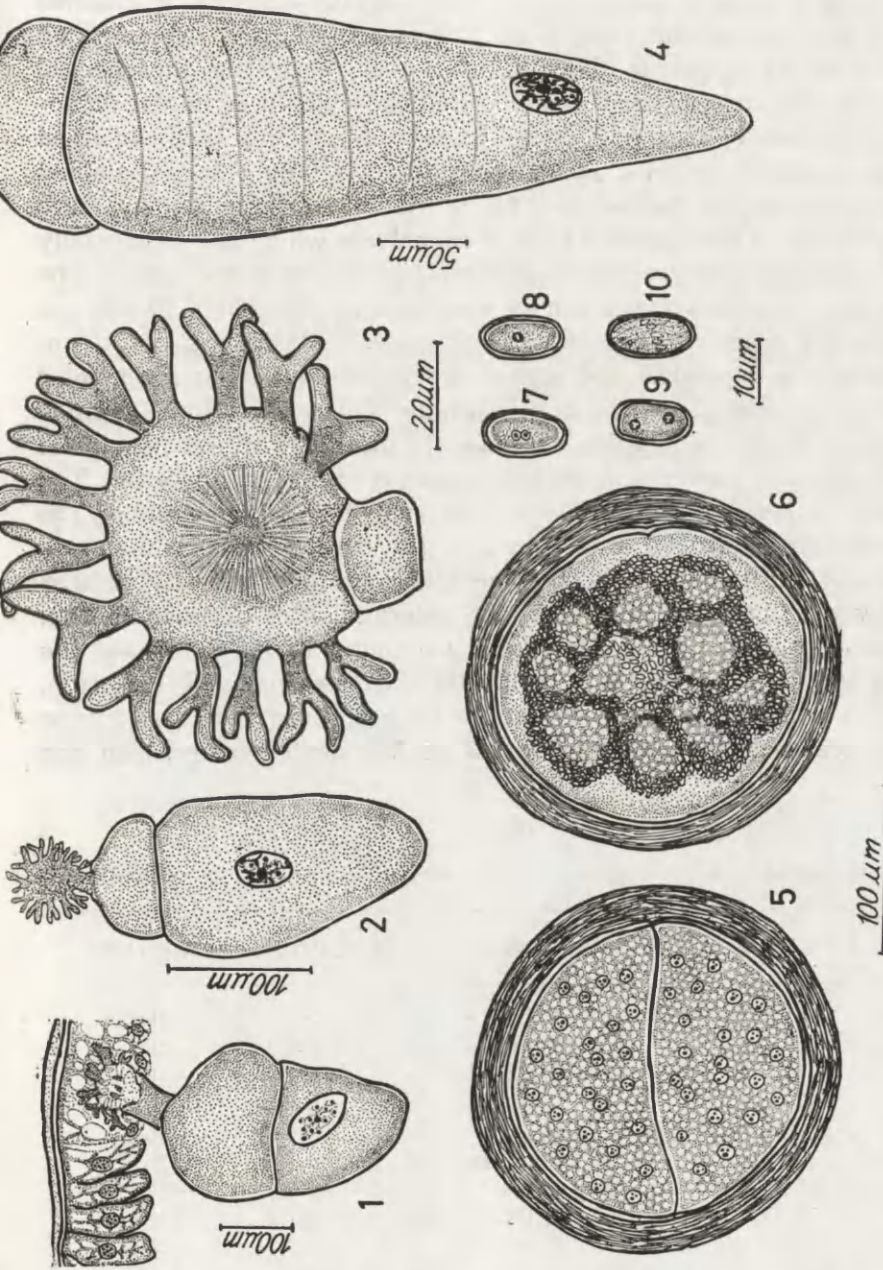
Fig. 1. *Dendrorhynchus keilini* sp. n. 1 — Attachment of a cephalont to midgut epithelium, 2 — A cephalont, 3 — Epimerite — Enlarged view, 4 — Sporadin, 5 — A cyst showing 20-25 nuclei in each gametocyte, 6 — Arrangment of sporoblasts on the periphery of cytoplasmic lobes, 7 — A zygote, 8 — Sporoblast with 1 nucleus, 9 — Sporoblast with 2 nuclei, 10 — A sporocysts showing the sporozoites

lected during February–May 1975 harboured a new species of a septate gregarine belonging to the genus *Dendrorhynchus* in the midgut.

The earliest stage of development of the parasite observed is attached to the midgut epithelium (Fig. 1 *1*). It consists of three segments, an anterior complex epimerite with a short neck, a middle subspherical protomerite and an elongated deutomerite. The cytoplasm in the deuto-merite is whitish opaque and dense when compared to that contained in the protomerite. An oval nucleus containing a single deeply stained endosome is present in the anterior 1/3 of the deutomerite. The structure of the epimerite is more clearly seen in cephalonts which are accidentally detached from the gut epithelium and are found free in the lumen. The fully grown cephalonts reach a maximum size of 200–400 × 70–130 μm (Fig. 1 *2*). The epimerite measures 40–64 μm × 20–32 μm and the tip of the epimerite is expanded into a disc-like structure having a diameter of 32.0 μm. The margin of the disc is produced into 13–16 bifid papillae (Fig. 1 *3*). Each of the papillae measures 16 μm × 4 μm and penetrates the epithelial cell partly and secures attachment for the parasite. The protomerite measures 104 × 130 μm and is widest in the middle. The deutomerite measures 230 × 130 μm and is broadest in the middle and tapers to a blunt point posteriorly. The epicyte is longitudinally striated and transverse fibrils are present in the anterior 2/3 of the deutomerite. The mature sporadins are solitary and are found in the lumen of the midgut (Fig. 1 *4*). They have a broad protomerite measuring 50 × 100 μm. The deutomerite measures 300–400 × 130–150 μm. Conspicuous transverse striations are seen in the anterior 2/3 of the deutomerite which are

Table 1

Measurements of sporonts of *Dendrorhynchus coromandelianum* sp. n. (in microns)

S. No.	L P	L D	W P	W D	T L	L P:T L	W P:W D
1	105	255	140	195	360	1:3.4	1:1.3
2	80	220	128	140	300	1:3.7	1:1.1
3	50	360	136	156	410	1:3.2	1:1.1
4	72	200	80	100	272	1:3.7	1:1.2
5	80	292	132	152	372	1:4.6	1:1.1
6	76	208	72	80	284	1:3.7	1:1.1
7	100	172	104	128	272	1:2.7	1:1.2
8	104	208	100	104	312	1:3	1:1
9	112	256	140	148	368	1:3.2	1:1
10	84	228	116	132	312	1:3.7	1:1.1

LP — length of protomerite, LD — length of deutomerite, WP — width of proto-merite, WD — width of deutomerite, TL — total length.

probably contractile fibrils. The Tl: Pl and Dw: Pw of sporadins of different sizes is given in Table 1.

Some of the sporadins found in the lumen of the hindgut are associated side by side with their protomorites directed away from each other, but the process of cyst formation could not be followed because the associated stages did not survive for more than about 30 min outside the body of the host. Cysts are found in the hindgut during April-May period. They are spherical having a diameter of 255 μm. There is an ectocyst 30 μm thick. Cysts passed out along with the faecal matter of the host show about 20–25 nuclei in each gametocyte (Fig. 1 5). When gametogenesis is completed, approximately about 48 h after the cysts are passed out by the host, the cysts appear transluscent instead of being opaque white as in earlier stages. Isogamous gametes are oval and measure 7.5 × 4.5 μm. The cytoplasm is hyaline and there is a deeply stained centrally placed nucleus. A distinct nuclear membrane has not been observed. When sporogony is completed the spores are arranged along the periphery of lobes of cytoplasm (Fig. 1 6). The nuclei of the gametes and zygotes could be stained by the usual staining procedures but when the sporoblast wall is formed most of the staining procedures were a failure. However, treating the spores according to Feulgen's technique stained the nuclei of the spores (Fig. 1 8, 9 and 10). Dehiscence is simple releasing spores which are oval measuring 12.0 × 5.0 μm. Sporozoites are spindle-shaped measuring 7.0 × 3.0 μm with a single deeply stained centrally placed nucleus. Residual protoplasm appears in the form of refringent granules in the centre of the sporocysts.

Taxonomic Position

The present gregarine qualifies for inclusion in the genus *Dendrorhynchus* Keilin because it posseses a disc-shaped epimerite with ramified papillae, longitudinally striated epicyte and transverse fibrils in the deutomerite. While describing *D. systeni* K e i l i n (1920) stated — "the body of the sporont is elongated with the posterior end slightly curved and of irregular contour, it does not seem to be divided into two segments, protomerite and deutomerite as is usual in cephaline gregarines". In the present form the cephalonts have three clearly demarcated segments and thus differs from *D. systeni*. The figures of *D. systeni* show that the papillae of the epimerite may have 2, 3 or 4 branches while in the present form the papillae are consistenly bilobed. The fully grown sporont in *D. systeni* measures 225.0 × 18.5–20.0 μm while in the present form it measures 400.0 × 150.0 μm. K e i l i n (1920) found subspherical cysts 60.0–80.0 μm in diameter in the larvae of *Systenus* sp. which unfortunately did not show the spores. He also encountered elongate cysts

measuring 100.0 × 40.0 µm which he assumed to be those of *D. systeni*. (K e i l i n was dealing with a mixed infection of a *Schizogregarine* resembling *Schizocystis gregarinoides* Leger, 1910 and *Taeniocystis mira* Leger, 1906). In the present case there is no mixed infection and hence the cysts collected from the faecal matter belonging to the only gregarine present in the gut.

The cysts in the present form have a diameter of 255.0 µm and an ectocyst 30.0 µm thick. The spores measure 12.0 × 5.0 µm and thus the present form differs from *D. systeni*. In view of all these differences the present form is considered a new species and the name *Dendrorhynchus keilini* sp. n. is proposed for the same in honor of late Professor D. Keilin, a distinguished protozoologist.

Ancyrophora ceriagrioni sp. n.

D i a g n o s i s. Cephalonts elongate, cylindrical measuring 900 × × 200 µm; Epimerite complex and in the form of a shallow cup, the margin of which is produced into 17–20 digitiform processes which are folded inwards when contracted. Sporonts solitary, cysts spherical 255–350 µm in diameter with an ectocyst 40 µm thick. Dehiscence simple, spores biconical with four polar spines, two at each pole; four equatorial spines, two on each side.

Eight out of 40 (20%) of adult *Ceriagrion coromadelianum* (Fabr.) collected during June–July harboured a new species of a septate gregarine belonging to the genus *Ancyrophora*.

Fully grown cephalonts were found in the lumen of the midgut. They are elongate, cylindrical and reach a maximum size of 900 × 200 µm (Fig. 1 *11*). The epimerite is in the form of a shallow bowl the margin of which is produced into 17–20 digitiform processes folded inwards like the petals of a bud (Fig. 2 *12*). When the epimerite is expanded the digitiform processes are unfolded and appear like the petals of a flower. The protomerite is broader than long measuring 90 × 245 µm and is in the form of a broad shallow cup. It is filled with coarsely alveolated lightly stained cytoplasm. The deutomerite measuring 765 × 200 um is elongate, cylindrical and is broadest anteriorly where it joins the protomerite. The nucleus is spherical and is situated in the anterior 1/3 of the deutomerite. It contains a single deeply stained centrally placed endosome. The sporonts reach a maximum size of 825 × 150 µm and are elongate and cylindrical (Fig. 2 *13*). Cysts are spherical and range in diameter from 250–350 µm and have an ectocyst 40 µm thick (Fig. 2 *14, 15*). Sporulation is completed in about 72 h after the cysts are passed out by the host and the dehiscence of the spores is by simple rupture
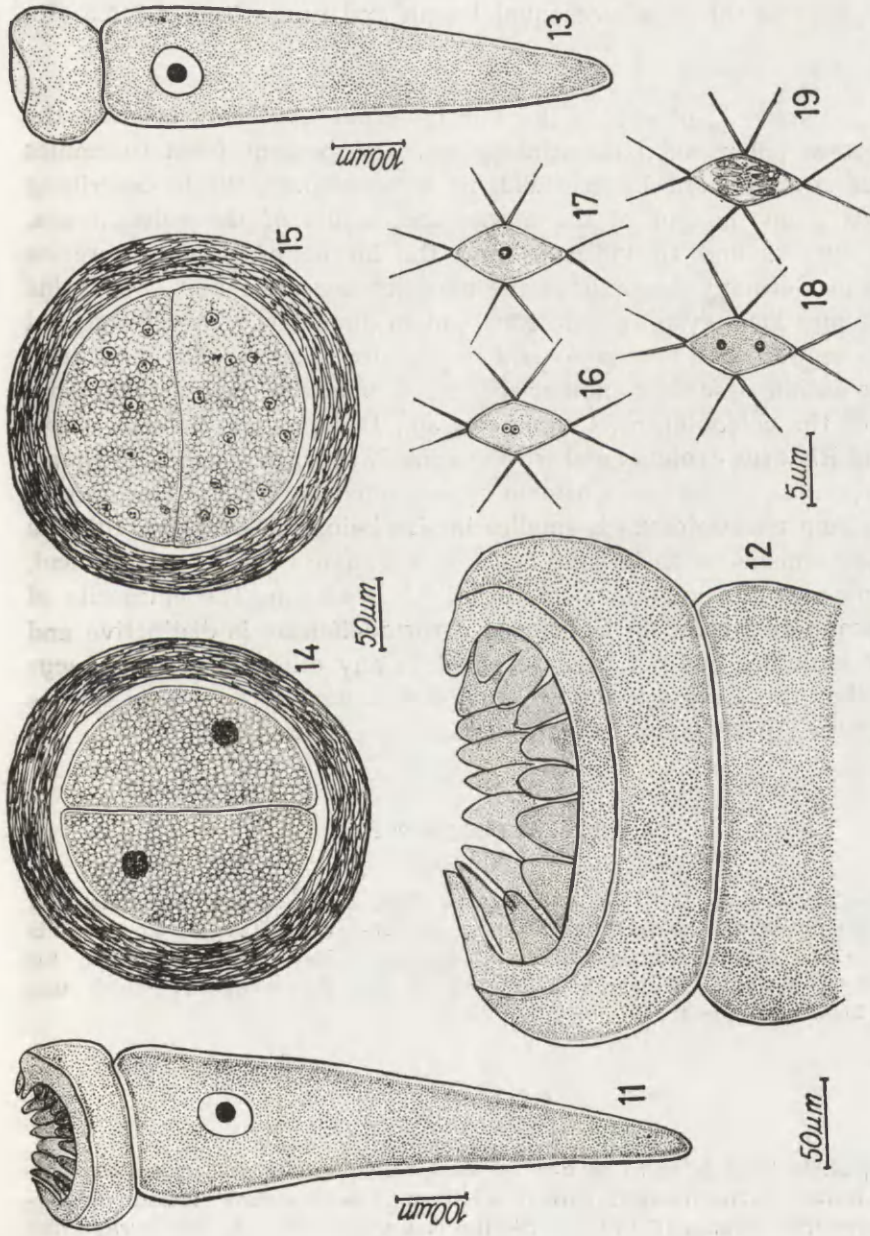
Fig. 2. *Ancyrophora ceriagrioni* sp. n. *11* — A cephalont, *12* — Epimerite enlarged, *13* — A sporont, *14* — A cyst showing 2 nuclei, *15* — A cyst showing several nuclei in each of the two gametocytes, *16* — A zygote, *17* — Uninucleate sporoblast, *18* — Binucleate sporoblast, *19* — A sporocyst showing sporozoites

of the cyst. Spores measuring 5.4–6.5 × 5.0–5.4 μm are biconical with two polar spines at each pole and two equatorial spines on each side (Fig. 2 17, 18, 19). The spore wall is thickened in places where the spines take their origin. The spines are of equal length and range from 4.5–5.5 μm.

Taxonomic Position

The gregarine is placed in the family *Acanthosporidae* because the spores posses polar and equatorial spines. The present form resembles the genus *Ancyrophora* Leger, 1892, in some respect. While describing *A. gracilis* from the gut of the larvae and adults of the coleopterans, *Carabus auratus* and *C. violaceus* and the larvae of *Silpha thoracica* collected in Germany Leger gave the generic characters as "...Sporadins 200 μm–2 mm long, cysts spherical 200 μm in diameter; spores hexagonal in optical section with four polar and six equatorial spines, 8.5 × 5.5 μm". The same author described another species, *A. uncinata* (Leger, 1892) from the gut of the coleopterans, *Colymbetes* sp., *Dytiscus* sp. *Noterus clavicornis* and *Rhantus exoletus* and trichopterns *Phryganea grandis*, *P. rhombica*, *Phryganea* sp. and *Sericostoma* sp. collected in France. This species reported from trichopterans is smaller in size being 150–200 μm with the epimerite garnished with 12 rigid hooks in alternate rows, cysts spherical, spores spined, both polar and equatorial 7.5 × 4.5 μm. The epimerite of the gregarine discovered in *Ceriagrion coromadelianum* is distinctive and a similar structure has not been reported in any other species of *Ancyrophora*. For these reasons the present form is considered a new species and the name *Ancyrophora ceriagrioni* sp. n. is proposed.

RÉSUMÉ

La morphologie et le cycle de deux espèces nouvelles des gregarines, *Dendrorhynchus keilini* et *Ancyrophora ceriagrioni* sp. n., trouvées dans l'intestin de *Ceriagrion coromandelianum* (Fabr.), est décrite. Les trophozoïtes de *Dendrorhynchus keilini* atteignent 400×300 μm au maximum. L'épimerite est developpé en forme de disque dont les marges produisent 13–16 papilles qui partiellement pénetrent dans les céllules de l'epithèle. Les kystes arrivent à 255 μm de diamètre et

comportent une ectocyste de 30 μm d'épaisseur. Les gametès isogamiques de 7.5×4.5 μm. Les spores ovales de 7.0×3.0 μm. Chez *l'Ancyrophora ceriagrioni* les trophozoïtes sont des dimensions de 900×200 μm et les sporontes de 825×150 μm. L'épimerite en forme d'un bol plat dont les merges produisent 13–16 protubérances digitiformes. Les kystes sphériques varient entre 255–350 μm de diamètre, avec une ectocyste de 40 μm d'épaisseur. La sporogonie se termine 72 h. Les spores biconiques, de dimensions de 6.5×5.4 μm avec 4 épines polaires et 4 équatoriales.

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compartant une couverté de ... déjà dans ... nombre ... épaisseur de
7.8×49 µm. Les épetit ... ovales en forme ... Chez ... épaisseur ...

L'épiderme en forme d'un col plat dont le mérite funéraire ...

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... The distance ... Longipalpus ...
... 126.

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Eimeria suncus sp. nov. (*Sporozoa: Eimeriidae*) from the Common House Shrew, *Suncus murinus murinus* Linnaeus)

Synopsis. In the course of survey of parasitic infection in the Common House Shrew, *Suncus murinus murinus* (Linnaeus) a few coccidian oocysts were recovered from the intestinal contents. On detailed examination it has been found as new and designate as *Eimeria suncus* sp. nov. The oocysts measure 18.2–21.8 µm (average 19.5 µm) by 15.5–16.8 µm (average 15.2 µm). Sporocyst lemon shaped, measuring 9.5–11.2 µm by 6.5–8.8 µm (average 10.5×7.8 µm). Sprozoites almost comma-shaped, each end is provided with a vacuole.

During the course of parasitic investigation on Insectivores, besides helminths, a coccidian parasite belonging to the genus *Eimeria* has been recorded from the Common House Shrew, *Suncus m. murinus* (Linnaeus) almost simultaneously from the two different localities (i) Mathura Veterinary College Campus and (ii) Singur, Hooghly, West Bengal. And on close investigation it has been found that both the materials are belonging to the same species, and does not resemble to any known species of the genus *Eimeria*, therefore described here as new. Moreover, the present report constitutes new host-parasite record as well as from a new locality. The type materials will be deposited to the National Collection of the Zoological Survey of India, Calcutta.

Material and Methods

About 50 specimens of *S. murinus murinus* were collected and examined from both the localities, of which 12 were found to harbour this parasite. The faecal samples were collected and left in 2.5 per cent potassium dichromate solution in Petri-dishes for sporulation. Sheather's sugar solution was used for concen-

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trating the oocysts by centrifugation (L e v i n e 1961). Morphological details of unsporulated and sporulated oocysts were studied under oil-immersion objective of an Olympus phase-contrast microscope with 15 × eye-piece lens. Sketches were made with the aid of a Spencer's camera lucida.

The scrapings from the different regions of small intestine, on examination, also revealed identical oocysts. The sections of the parts of small intestine, stained with haematoxyline and eosin, showed characteristic oocysts as well as mature macrogametes and microgametocytes.

Description

Eimeria suncus sp. nov.

Oocysts (50 measured) almost subspherical (Fig. 1) measuring 18.2 µm– 21.8 µm in length with a mean of 19.50 µm by 15.5–16.8 µm in width with a mean of 15.2 µm. It is provided with smooth bilayered wall of yellowish in colour measuring 1.9 to 1.65 µm in thickness and the outer being thinner. A clear micropylar cap is visible on the wall of the oocyst but no distinct micropyle is seen in any of the oocysts examined. Oocystic residuum and polar granules absent. Sporocysts lemon-shaped, measuring 9.5–11.2 µm in length with a mean 10.5 µm and 6.5–8.8 µm in width

10µm
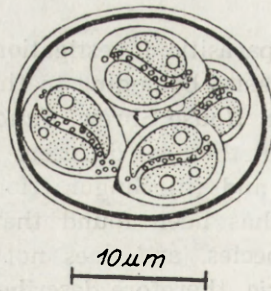
with a mean of 7.8 µm. It is provided with a nipple like protuberance having a slightly thickned area at one end. Sporocystic residuum appeared as beaded like globular mass scattered in side the sporocyst. Sporozoites are almost comma-shaped measuring 4.00–5.5 µm in length with a mean of 4.5 µm and 3.2–3.8 µm in width with a mean of 3.5 µm. Each end of the sporozoite is provided with a clear refractile area and the bigger one is found at the wider end.

Type host: *Suncus m. murinus* (Linnaeus)
Seat of infection: Intestine.
Type locality: Mathura Veterinary College Campus, U. P.
Holotype: Z. S. I. Registration No.

Endogenous stages

The stained sections exhibit oocysts, macrogametocytes and micro-gametocytes inside columnar epithelial cells. The oocysts in sections were almost of the same size and shape as those recovered, from the intestinal contents. They measured 21.26–20 μm × 12–18.6 μm in size. The macrogametocytes with large peripheral plastic granule measuring 14 μm by 12.56 μm have been noticed. The schizonts could not be located.

Remarks

The present species resembles in shape and size with *E. milleri* Bray, 1958, *E. soricis* Henry, 1932 and *E. crociduriae* Galli-Valerio, 1933 occurring in the genera *Crocidura* and *Sorex* but can easily be separated from all of them in having a distinct micropylar cap on the wall of the oocyst. It also comes to *E. dissimilis* Yakimoff and Gousseff, 1935 of *Sorex araneus* due to the presence of micropyle/micropylar cap but differs in having polar granules by the latter. Therefore, the species reported here in does not resemble to any known species described so far and named as *Eimeria suncus* sp. nov. Further this species represents the first record of its kind in *Suncus m. murinus* (Linnaeus) and reported for the first time from an insectivorous host of the Indian subregion.

RÉSUMÉ

En examinant l'infection parasitaire chez *Suncus murinus murinus* (Linnaeus) on a récupéré du contenu de l'intestin des peu nombreuses oocystes de coccidie. Leur étude détaillée a montré qu'elles appartiennent à une espèce nouvelle qui a été désignée comme *Eimeria suncus* sp. n. Les dimensions des oocystes varient entre 18.2–21.8 μm (19.5 μm en moyenne) sur 15.5–16.8 μm (15.2 μm en moyenne). Les sporocystes, en forme de citron, varient entre 9.5–11.2 μm sur 6.5–8.8 μm (10.5×7.8 μm en moyenne). Les sporozoïtes, en forme de virgule, sont munis d'une vacuole à chaque extrémité.

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Isospora concinnus sp. n. from a Sikkim Red-headed Tit, Aegithalos concinnus rubricapillus (Ticehurst)

Synopsis. A new coccidium *Isospora concinnus* sp. n. (*Protozoa: Eimeriidae*) is described from faecal sample of a Sikkim red-headed tit, *Aegithalos concinuus rubricapillus* (Ticehurst). Its affinities with the known species of the genus and differences to consider it as new species have been also incorporated.

This is the second instalment of the series deals with the coccidian parasite of a Himalayan bird. Literature on avian coccidia from Himalayan region reveals that a few species of *Isospora* have been described so far from India. R a y et al. (1952) reported five new species viz: *Isospora coraviae* from *Corvus macrorhynchus intermedius* the common Himalayan crow, *I. seicercussae* from *Seicercus xanthoschistos* the greyheaded fly catcher-warbler, *I. garrulae* from *Garrulax lineatus lineatus* the streaked laughing thrush, *I. garrulussae* of *Garrulus glandarius bispecularis* the Himalayan Jay and *I. parusae* from *Parus dichrous* the brown crested tit, obtained at Mukteswar (altitude. 2460 m). S i n h a et al. (1978) have recorded one species of *Isospora* from North-eastern Himalayan region.

This paper includes the description of a new coccidium, *Isospora concinnus* sp. n. from *Aegithalos concinnus rubricapillus* (Ticehurst) collected at Darjeeling (altitude 2076 m), West Bengal, India. The specific name of the parasite is being given after the specific name of the host bird.

Material and Methods

Faecal samples of six red-headed tits were examined microscopically in saline solution and three were positive for coccidian oocysts. For preservation and study of development of oocysts faecal content was kept in a Petri dish mixed with 2.5% potassium dichromate solution and the measurements of the oocysts were

taken. One heavily infected bird was sacrificed for studying endogenous phases of the parasite. Small pieces of tissue were cut from the intestine and fixed in Carnoy's fixative. The tissues were paraffin embedded and sectioned at 5 µm and then stained with haematoxylin and eosin as well as Heidenhains-iron-haematoxylin method. The diagrams represented here drawn with the help of a prism type camera lucida. The magnification was 1500 × in all cases.

Results

Isospora concinnus sp. n.

Type host. *Aegithalos concinnus rubricapillus* (Ticehurst)
Type locality. Darjeeling (altitude 2076 m), West Bengal, India.
Site of infection. Small intestine.
Sprulation time. 24–36 h.

Schizogony. It takes place in the small intestine of the host. Trophozoite (Fig. 1 *1*) is more or less oval in shape with a thin outer covering measuring 7 µm × 4.2 µm. A distinct nucleus is present. Schizonts are oval or circular with a number of nuclei. A mature schizont (Fig. 1 *2*) measures 13.8 µm in diameter and contains 16 nuclei with a developing merozoite which measures 5.3 µm in length.

Gametogony. A large number of macrogametes are encountered in the serial sections of the small intestine, usually in epithelial cells of the mucous membrane often subepithelially in the muscularis mucosae.

Early macrogamont (Fig. 1 *3*) is oval measuring 9.8 µm × 8.4 µm. The cytoplasm is alveolar in nature and contains a few deeply basic stained dots or granules. A centrally placed nucleus with a distinct nuclear membrane is present. A halo is found encircling the karyosome which stains blue black in iron-haematoxylin stain and occupies the most of the part of the nucleus. The growing macrogamont is spherical or oval. A fully formed female gamont (Fig. 1 *4*) measures 16.8 µm × 15.4 µm.

The microgamonts are round or oval bodies found abundantly in the sections. The young microgamont (Fig. 1 *5*) 7.4 µm in diameter, has a large number of nuclei which have got blue black colour in iron-haematoxylin method stain. A mature spherical shaped male gamont attains 14.8 µm in diameter. Te fully developed one is seen to contain microgametes which are small comma shaped bodies.

Sporogony. Oocysts are spherical ranging from 23.5 µm to 29 µm × × 21 µm to 25 µm with a mean of 27 µm × 23 µm. The oocystic wall
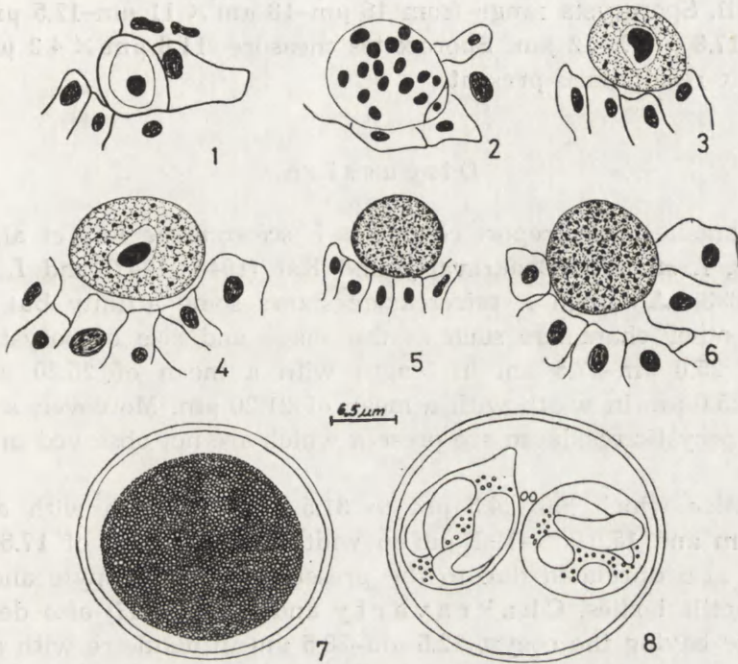
Fig. 1 *1–8*. Camera lucida drawings of *Isospora concinnus* sp. nov. *1* — An early trophozoite, *2* — A multinucleated schizont with a developing merozoite, *3* — An early macrogamont, *4* — A mature female gamont, *5* — A young microgamont, *6* — A mature malegamont containing microgametes, *7* — An unsporulated oocyst in faecal content, *8* — A sporulated oocyst with two sporocysts each containing four sporozoites in faecal content (Figs. *1–6,* in the section of small intestine)

is smooth, bilayered with a uniform thickness of 2 μm. An oocystic residuum or a micropyle or a polar granule is absent. One or two refractile bodies are found lying between the outer sporocystic walls.

Fully grown sporocysts are conical in shape. The anterior end is to some extent pointed while the posterior part is almost rounded. Each of two sporocysts has four sporozoites. A mature sporocyst (Fig. 1 8) are elongated bodies which overlap each other with in the sporocysts. They measure 11.8 μm in length and 4.2 μm in breadth.

Diagnosis of *Isospora concinnus* sp. n.

Oocysts measure 23.5 μm – 29 μm × 21 μm – 25 μm with a mean 27 μm × 23 μm. There is neither a micropyle nor an oocystic residuum but one or two refractile bodies are seen to lie between the outer sporo-

cystic wall. Sporocysts range from 16 μm–18 μm × 11 μm–12.5 μm with a mean 17.8 μm × 12 μm. Sporozoites measure 11.8 μm × 4.2 μm. The sporocystic residuum is present.

Discussion

The parasite under report resembles *I. scicercussae* Ray et al. (1952) *I. muniae, I. sturniae* Chakravarty and Kar (1944, 1947) and *I. lacazei* Labbe (1893). Although *I. seicercussae* shows some affinity but differs in many other characters such as the shape and size of oocyst which measures 25.0 μm–27.5 μm in length with a mean of 25.20 μm and 20.0 μm–25.0 μm in width with a mean of 21.20 μm. Moreover, a micropyle and oocystic residuum are present which are not observed in *I. concinnus*.

I. muniae which has 24.5 μm to 31.5 μm in length with a mean of 28.3 μm and 15.5 μm–19.5 μm in width with a mean of 17.5 μm of oocyst is also unrelated due to the presence of a micropyle and lacks any refractile bodies. Chakravarty and Kar (1947) also described *I. sturniae* having the oocyst 22.5 μm–28.5 μm in diametre with a mean of 25.5 μm. A micropyle is present in case of it. Oocyst of *I. lacazei* differs considerably from the new species described here because in former the length of the oocyst varies from 16 μm–30 μm and the sporocyst contains a large residual body which are sufficiently different in *I. concinnus* later form and moreover *I. concinnus* does not show such variation in measurements.

Thus, the present coccidium does not fit with any known species and hence it is described here as new species.

All the type specimens will be submitted to the National Collection of the Zoological Survey of India, Calcutta, in due course.

ZUSAMMENFASSUNG

Aus Kotmaterial eines "Sikkim Red-Headed Tit" *Aegithalos concinnus rubricapillus*, (Ticehurst) wurde *Isospora cancinnus* sp. n. isoliert. Der Vogel, der Wirt, wurde in der Bergstadt Darjeeling (H. 2076M) im Ost-Himalaya, Indien, gefangen. Die charakteristischen Merkmale der neuen *Isospora* sp. sind beschrieben worden.

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Jerzy J. LIPA

Nosema peridromae sp. n., a New Microsporidian Parasite of the Variegated Cutworm *Peridroma saucia* (Hbn.) (*Lepidoptera, Noctuidae*)

Synopsis. A new microsporidian *Nosema peridromae* sp. n. is described from a noctuid *Peridroma saucia* (Hbn.) collected in France and in USA. Binucleate and tetranucleate schizonts were observed. Sporonts were binucleate. Spores were oval and measured 1.9–3.2×1.5–2.1 μm. The parasite caused general infection.

Several microsporidians have been recorded in noctuids (S p r a g u e 1978). In this paper I report on a new microsporidian from the variegated cutworm *Peridroma saucia* (Hbn.) (= *Peridroma margaritosa* Hbn.), known under several synonyms and having a worldwide distribution as an economic pest of various cultivated plants (R i n g s et al. 1976).

Material and Methods

Infected larvae and pupae of *Peridroma saucia* were first observed in insectary of Station de Zoologie Agricole INRA in Montfavet, on November 29, 1969. During my next visit I collected on September 28, 1972 some infected larvae on tomato plantation at Thor near Montfavet.

Various tissues of larvae were microscopically examined and in case of infection smeares were prepared. These were air dried, fixed in methanol and then stained with Giemsa's 0.25⁰/₀ solution for 24 h.

Results

Nosema peridromae sp. n.

Host: *Peridroma saucia* (Hbn.)
Infected tissues: General infection, fat body and midgut were most heavily attacked.
Locality record: 29.IX.1969 Montfavet (France) and 28.IX.1972, Lincoln County (Nebraska, USA) on 16.VI.1958.

Vegetative Stages

The youngest uninucleate schizonts were 2 to 3 µm in diameter. Older schizonts were binucleate (Pl. I 1,2) and tetranucleate (Pl. I 3, II 4) and had 4 to 6.5 µm in diameter. Chains of schizonts were not observed.

Sporulation

Sporonts were elongated and the largest ones measured 6.5 by 2 µm. They had two nuclei (Pl. I 3) and were weakly stained with Giemsa's stain as compared to schizonts. Since a binucleate sporont divides into two sporoblasts maturing into spores this microsporidian belongs to the genus *Nosema*.

Spore

Spores were oval. Fresh spores in water measured 2.8–3.3 $\times$ 1.8–2.2 µm. Fixed and stained spores (Pl. II 5) measured 1.9–3.2 $\times$ 1.5–2.1 µm (Table 1).

Table 1

Comparison of frequency distribution of length of two samples of fixed and stained spores of *Nosema heliothidis* L. et S. and *Nosema peridromae* sp. n.

Microsporidian	Spore size [µm]						
	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0
Nosema peridromae sp. n.	7	50	33	10			
Nosema heliothidis L. et S.			1	12	23	11	3

Tissues Attacked

The parasite attacked all tissues and caused general infection. The fat body and midgut epithelium were most heavily attacked.

Infection Level

In insectary rearing of *P. saucia* at Station de Zoologie Agricole 1NRA at Montfavet out of nine examined larvae on November 28, 1969 three of them were infected with *Nosema* sp. Among six larvae collected in the field on September 28, 1972 at Thor only one larva was infected.

Taxonomic Position

The studied *Nosema* sp. caused general infection similar to that caused by *Nosema heliothidis* L. et S. in *Heliothis* spp. (Lipa 1968). However, the size of spores of both species greatly differ (Table 1)

what indicates that these two microsporidians are different species. Other microsporidians known from *Noctuidae* have different development and larger spores or attack other tissues of their hosts (S p r a g u e 1978). Therefore, I consider that *Nosema* sp. found in *Peridroma saucia* is a new species and propose a name *Nosema peridromae* sp. n. for it.

L i p a and S t e i n h a u s (1962) and S t e i n h a u s and M a r s h (1962) reported from a larva of *Peridroma saucia* (= *Peridroma margaritosa*) collected in Nebraska a microsporidian infection caused by *Nosema* sp. Unfortunately, no data on site of infection, development or spore size of this microsporidian are available but apparently it was a first record of *Nosema peridromae* sp. n. described in this paper.

RÉSUMÉ

Nosema peridromae sp. n. est une nouvelle espèce du protozoaire parasite trouvée chez les chénilles de *Peridroma saucia* (Hbn.) récoltées en France en 1969 et 1972. Le parasite provoque une infection générale, mais il attaque en particulier l'intestin et corps adipeux. Dans son cycle de développement on trouve des schizontes à deux et à quatre noyaux. Les sporontes sont de forme ovale et leurs dimensions se situant entre 1.9–3.2×1.5–2.1 μm. Le même parasite fut trouvé aussi en USA chez les chénilles de *Peridroma saucia* (= *P. margaritosa*).

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what indicates that these two microsporidians are different species. Other microsporidians known from Noctuidae have different development and larger spores or attack other tissues of their hosts (Sprague 1978). Therefore, I consider that Nosema sp. found in Peridroma saucia is a new species and propose a name Nosema peridromae sp. n. for it.

Lipa and Steinhaus (1959) and Steinhaus and Marsh (1962) reported from a larva of Peridroma saucia (= Peridroma margaritosa) collected in Nebraska a microsporidian infection caused by Nosema sp. Unfortunately, no data on site of infection, development or spore size of this microsporidian are available but apparently it was a first record of Nosema peridromae sp. n. described in this paper.

RÉSUMÉ

Nosema peridromae sp. n. est une nouvelle espèce du microsporidie parasite trouvée chez les chenilles de Peridroma saucia (Hbn.) récoltées en France en 1959 et 1972. Le parasite provoque une infection généralisée du corps graisseux, l'intestin et corps adipeux. Dans son cycle de développement on trouve des schizontes à deux et à quatre noyaux. Les sporontes sont de forme ovale et leurs dimensions se situant entre 4.6–5.8 × 1.9.1 μm. Le même record fut trouvé aussi en USA chez les chenilles de Peridroma saucia (= P. margaritosa).

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EXPLANATION OF PLATES I–II

Nosema peridromae sp.n.

1: Binucleate schizont (Sc) and spores (S)
2: Uninucleate (Sc[1]) and binucleate (Sc[2]) schizonts
3: Schizonts (Sc), sporont (Sp) and spores
4: Tetranucleate (Sc[4]) and sporonts (Sp)
5: Spores in smeared tissues

PLATE I

J. J. Lipa auctor phot.

PLATE II
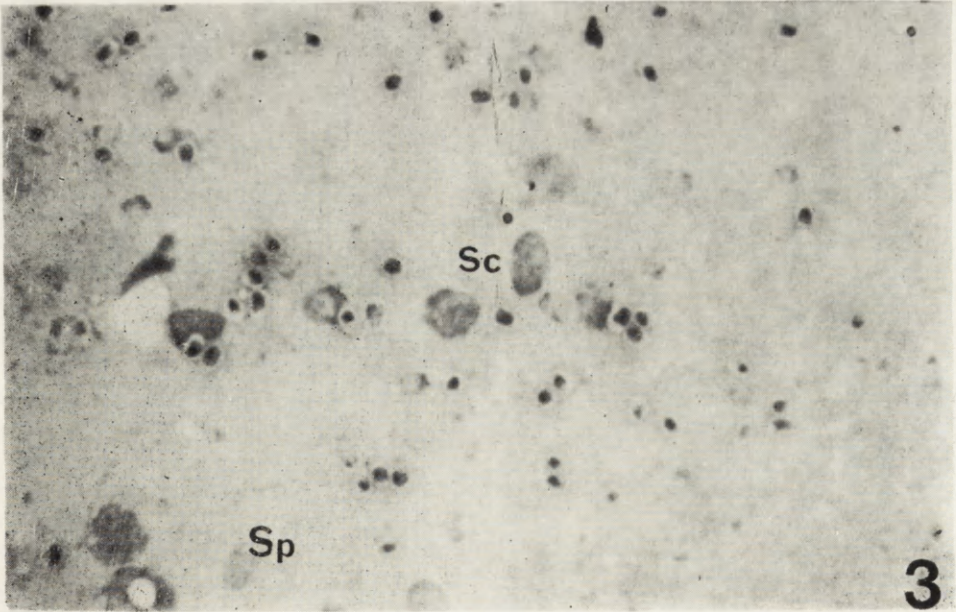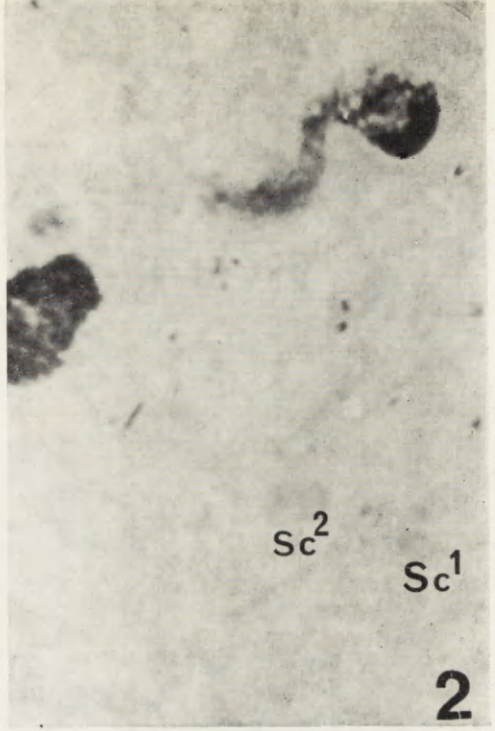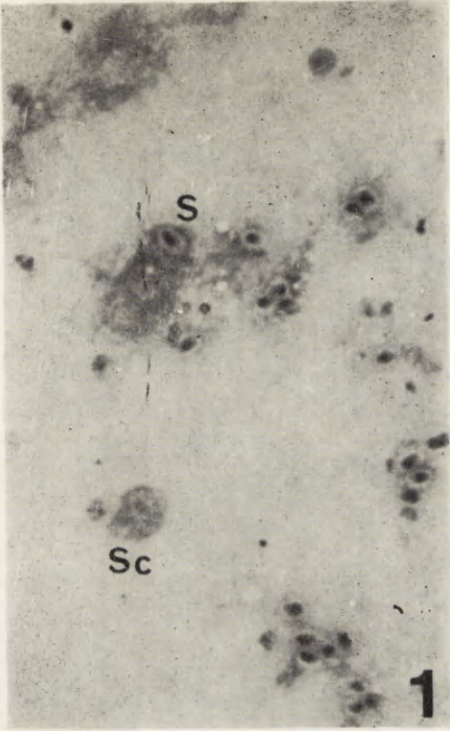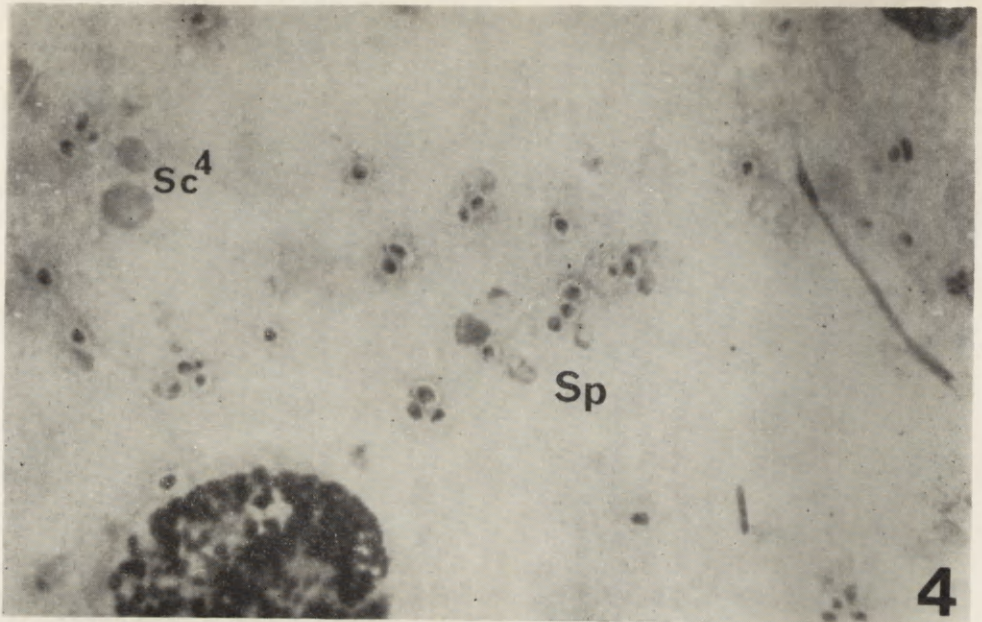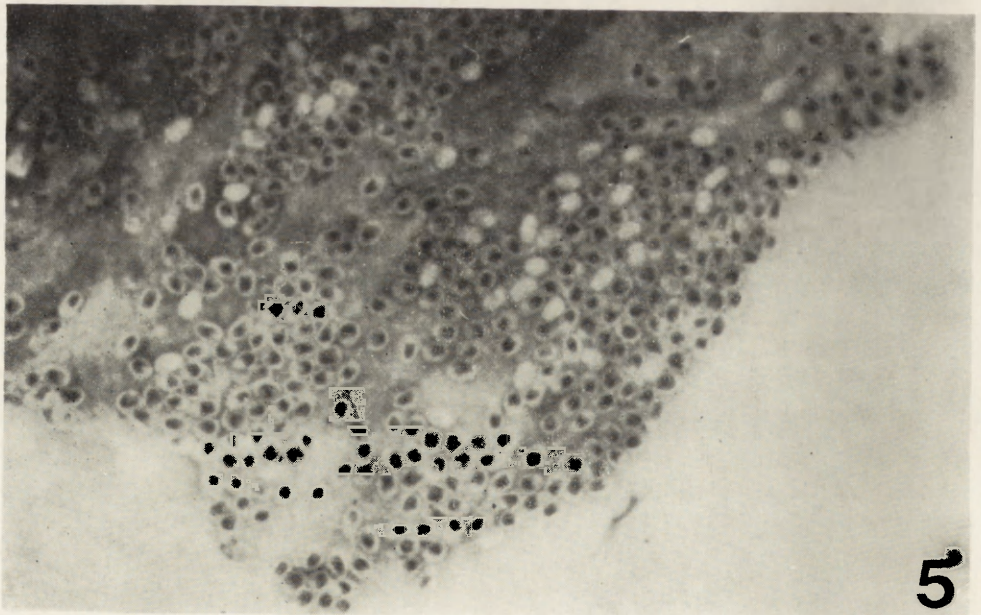
J. J. Lipa

auctor phot.

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S. A. PODLIPAEV and D. V. OSSIPOV

Early Stages of Infection of *Paramecium caudatum* Micronuclei by Symbiotic Bacteria — Omega-particles (Electron Microscope Examination)

Synopsis. Ultrastructural changes in *Paramecium caudatum* cells at early stages of infection of the micronuclei (MI) by symbiotic bacteria — omega-particles are studied. It has been found that the symbionts from culture media penetrate cells through food vacuoles. Further penetration to the cytoplasm is due to disruption of vacuole membrane. A wide zone of fibrillar material is formed in the cytoplasm around omega-particles, being connected with particular cylindrical strings, about 50 nm in the diameter. These structures are in close contact with MI envelope and, probably, perform a defined role, securing specificity of the infection. In the zone of contact with omega-particles the MI envelope forms a system of lobes, projections and depressions with, unusual for MI of ciliates, long inner and outer nuclear canals and finger-like projections comprising microtubules. The processes of MI envelope, directed to the omega-particles, merge together forming closed chambers around the bacteria, thus protecting MI karyoplasm from the contact with cytoplasm of ciliates. It is possible that in the process of penetration of omega-particles into the nucleus, the bundles of longitudinal microtubules play an important role. Single cases of nonspecific localization of the omega-particles in the macronucleus (MA) have been noted. Within one hour after infection zones enriched with cytoplasmic reticulum, appear in the cytoplasm. The results are compared with earlier data on sluicing the symbiotic jota-particles during their penetration into MA (Ossipov and Podlipaev 1977, 1978).

During last years the attention of investigators has been more and more turned to various forms of endonuclear symbionts of ciliates (Ball 1969, Beale et al. 1969, Preer et al. 1974). Symbiotic bacteria were found in various groups of *Ciliophora*, however, experimental investigations have been carried with only few species. In ciliates *Paramecium caudatum* the symbionts of the macronucleus (MA) — jota-par-

ticles (*Holospora obtusa* Hafkine, 1890), as well as of the micronucleus (MI) — omega-particles (*H. undulata* Hafkine, 1890) have been already described (O s s i p o v et al. 1976). Omega-particles, as well as jota-particles and many other symbiotic bacteria of paramecia are Gram negative organisms (P r e e r et al. 1974).

In the life cycle of omega-particles two morphological forms alternate: spindle-shaped, transversely dividing forms (length 1.5–2.0 μm) and spiral, spore-like ones (up to 12 μm), performing the function of dispersion of the symbiont and infecting intact ciliates. At all stages of the life cycle the cells of symbionts are lacking the active movement capacity. Some cyto-physiological peculiarities of intranuclear symbiosis in ciliates, have been discussed in detail in a series of papers (O s s i p o v et al. 1976, O s s i p o v and P o d l i p a e v 1976, 1977, 1978). The present paper is devoted to not yet investigated developmental stage of omega-particles and to the infection of *P. caudatum* MI. Investigations of the process of infection and intracellular transport of the bacteria are of great interest due to specific localization of all internal symbionts of paramecia that have been studied up to day.

Unfortunately there are only a few experimental researches concerning the mode of penetration of symbiotic micro-organisms into the nuclei of host cells. In fact, they are presented by the data on jota-particles in the ciliate *P. caudatum* and to the results of our present investigations.

Extension of our knowledge on intracellular translocation of foreign structures, which do not perturbate nuclear functions, is of great interest for understanding various forms and mechanisms of nucleus–cytoplasm relationships.

Material and Methods

The work was carried on *P. caudatum* ciliates of the clone MM-496-49a3 infected by omega-particles originating from not purified homogenate of paramecia, clone M-496-17-omega. Methods of culturing and infection of ciliates have been described earlier (O s s i p o v and I v a h n j u k 1972). One hour after homogenate had been added to the culture the paramecia were washed in sterile portions of culture medium. This procedure greatly contributed to obtaining rather synchronized infection. In order to increase the probability of finding MI in ultrathin sections the clone MM-496-49a3 was used, each cell of which contains three MI of the wild type in contrast to common clones of *P. caudatum* with only one MI. Special thanks are due to I. I. Skoblo for performing the infection of pure cells of *P. caudatum* with omega-particles.

Paramecia were fixed in 1–4 h from the beginning of infection. They were fixed in 2% OsO_4 in the cacodylate buffer (pH 7.4), then the material was em-

bedded in araldite. Sections were made with the aid of LKB-III ultramicrotome and were additionally contrasted with uranyl-acetate and lead citrate. They were examined in either Hitachi HU-11E or Tesla BS-500 electron microscopes operating at 75 kW or 60 kW, respectively. A part of cells was simultaneously stained by the methods of F e u l g e n or D i p p e l l (1955) for light microscope observations.

Results

The life cycle of symbionts in paramecia begins from swallowing of infective "spores" from outer environment (Pl. I 1). In that way omega-particles, together with food, get into food vacuoles of paramecia (Pl I 2–5). No essential changes in the bacteria organization during they stay in food vacuoles have been observed. The structure of symbionts at this stage of the life cycle corresponds with cyto-differentiation of spores in stably infected MI (G r o m o v et al. 1975) and in outer environment (Pl. I 1). Protoplasmatic body of the bacteria is irregular in shape and occupies 1/2 to 2/3 of the spore volume (Pl. I 2–5). The rest of the spore is filled by hypertrophied periplasmatic layer of the bacteria wall.

Within one hour after homogenate addition the number of cysterns of endoplasmatic reticulum in the ciliate cytoplasm highly increases (Pl. II 6). Their aggregations usually are situated in outer layer of the endoplasm.

The moment of getting out of omega-particles from food vacuoles has not been observed; probably the bacteria leave the vacuole very quickly disrupting its membrane (Pl. II 7). As a result, the symbiotic bacteria, together with food particles (*Aerobacter aerogenes*), are found free in the cytoplasm of paramecium. In sections it has not been possible to establish the moment of getting out of the protoplasmatic body (euinfective stage) from the spore: it is still not known whether this process takes place in the food vacuole or in the cytoplasm of paramecium. However, it is worth to mention that in all subsequent stages the structure of the cell wall and cytoplasm of the symbiont shows that the nucleus is infected by the omega-particles at the euinfective stage, measuring not less than 7–8 μm. Around the bacteria, in the cytoplasm, a granular layer up to 28 nm thick is formed. The granules are arranged in one or two layers; the inner one, about 8 nm, and the outer layer up to 13 nm thick (Pl. III 8 a).

Around most omega-particles occurring near MI a wide zone without clear borders appears (200–400 nm), being formed probably of a fibrillar material (Pl. III 9 a, b, Pl. IV 10 a–Pl. V 12 a). In the proximity

of omega-particles the density of the fibrillar material increases and an electron dense cover is formed. The above-mentioned granular cover may be found in close contact with the wall of bacteria (Pl. IV 10 a, Pl. V 12 a).

Connection of the fibrillar zone surrounding the bacteria with peculiar cylindrical strings, arranged in the cytoplasm of paramecium (Pl. Ill 9 a–c, Pl. IV 10 a–11, PI. V 12 a–13), attracts attention. These strings are in form of slightly curved cylinders with apparent periodic fibrillar structures. In particular sections these strings attain 300 nm in length and about 50 nm in the diameter (Pl. IV 10 b, d, 11, PI. V 12 b, c). There are also observed some structures forming a network (Pl. Ill 8 b, Pl. V 12 a). It is possible that these two types of strings, those of the network and the fibrillar ones, are genetically related. It cannot be excluded also that they represent functional differentiation of the same structure. In some cases we have found these strings in a close contact with MI envelope (PI. IV 10 a, d, Pl. V 12 a, c, 13). We do not know any other cases of similar structures being observed in the cytoplasm of paramecia or any other ciliates (J u r a n d and S e l m a n 1969, E h r e t and M c A r d l e 1974, V i v i e r 1974, O s s i p o v et al. 1976, O s s i p o v and P o d l i p a e v 1977, 1978). Around omega-particles, laying in the proximity of MI, occur aggregations of small vacuoles and canals of reticulum (Pl. Ill 8 a, Pl. IV 10 a, Pl. V 12 a).

It is worth to mention that the membranes of MI envelope in infected paramecia form a system of various invaginations and projections (PI. Ill 8 a, PI. IV 10 a, Pl. V 13, Pl. VI 14–21, Pl. VII 22–25). This feature distinguishes them from noninfected MI as well as from the nuclei stably infected [1] with omega-particles (O s s i p o v et al. 1976) and from MI of cells, the MA of which are at various stages of jota-particles infestation (O s s i p o v et al. 1976, O s s i p o v and P o d l i p a e v 1977, 1978). The invaginations of MI envelope are of various depth, from shallow ones up to deeply going into the nucleus (Pl. IV 10 a, Pl. VI 14, Pl. XII 35 a). The projections are formed by the outer membrane (Pl. IV 10 a, Pl. VI 16) as well as by both membranes of the nuclear envelope (Pl. V 13, Pl. VI 17–21, Pl. VII 22–25). Two types of changes of the nuclear envelope, being not yet observed in paramecia, attract special attention. First of them are represented by bent canals running towards the cytoplasm as well as inside the nucleus (Pl. VI 20, 21, Pl. VII 22, 23, 25, Pl. X 31, 32); some of the intranuclear canals bear peculiar microtubules. The second type is represented by finger-like projections with narrowings in proximal parts (Pl. VI 17,

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[1] Infection being maintained in a series of agamic cell generations.

Pl. XII 35 a), the latter being frequently provided with microtubules (Pl. VI 17).

The omega-particles penetrate the MI of paramecia not in any place but most preferably they chose the achromatine cap (Pl. IlI 8 a, 9 a–c, Pl. IV 10 a, Pl. XI 33, 34, Pl. XII 35 a). The analysis of electronograms, especially of succeeding sections (Pl. Ill 9 a–c), as well as light microscope investigations, show that the bacteria approach the MI surface at acute angle. Thus the process of penetration of symbionts into the nucleus has been studied step by step (Figs. 1, 2).
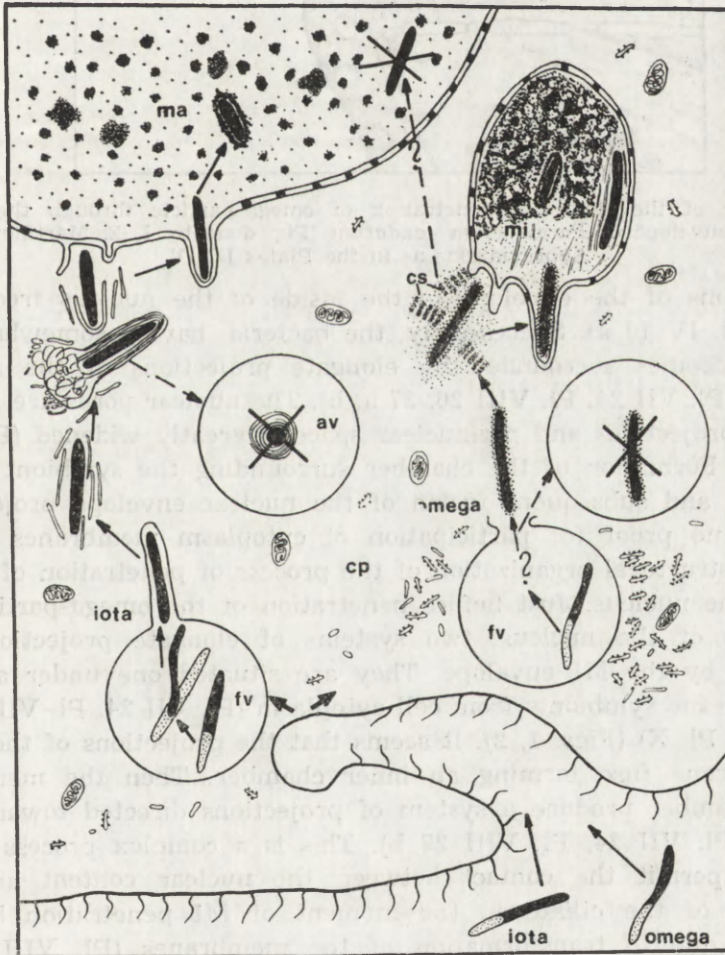
Fig. 1 a–f. Scheme of the sequent stages of changes in micronuclear envelope of *Paramecium caudatum* during the penetration of omega-particle ("Sluicing" of symbiont) Abbreviations as in the Plates I–XIV

A series of changes of the nuclear envelope begins from formation of the system of nuclear projections directed towards the bacteria (Pl. Ill 8 a, Pl. IV 10 a). At the place of contact of the omega-particles with the nuclear envelope the perinuclear space locally dilates and
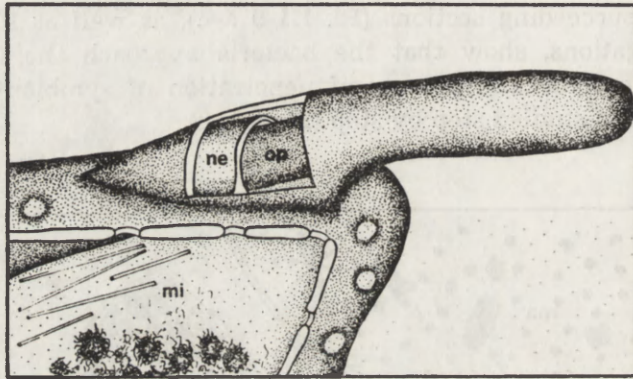
Fig. 2. One of the stages of penetration of omega-particle through the micro-nuclear envelope of *Paramecium caudatum* (Fig. done by I. E. Martinovitch) Abbreviations as in the Plates I-XIV

invaginations of the envelope to the inside of the nucleus frequently appear (Pl. IV 10 a). Successively, the bacteria, having somewhat bent outline, becomes surrounded by elongate projections of the nuclear envelope (Pl. VII 24, Pl. VIII 26, 27 a, b). The nuclear pores are lacking on these projections and perinuclear space is greatly widened (Pl. VIII 26, 27 b). Formation of the chamber surrounding the symbiont is due to growth and subsequent fusion of the nuclear envelope projections. We have no proof for participation of cytoplasm membranes of the ciliate in structural organization of the process of penetration of bacteria into the nucleus. Just before penetration of the omega-particles to the inside of the nucleus, two systems of elongate projections are developed by the MI envelope. They are situated one under another and isolate the symbionts from cell cytoplasm (Pl. VII 24, Pl. VIII 27 b, Pl. IX 29, Pl. X) (Figs. 1, 2). It seems that the projections of the outer MI membrane fuse forming an inner chamber. Then the membrane of this chamber produce a system of projections directed towards the bacteria (Pl. VII 24, Pl. VIII 27 b). This is a complex process which does not permit the contact between the nuclear content and the cytoplasm of the ciliate in the moment of MI penetration by the symbiont, due to transformation of the membranes (Pl. VIII 27 a, Pl. IX 30, Pl. X 31, 32, Pl. XI 33), resembling the process of sluicing of jota-particles penetrating the MA of *P. caudatum* (O s s i p o v and P o d l i p a e v 1976, 1977).

The perinuclear space is greatly widened in a zone of penetration of MI by the bacteria (up to 100–150 nm), however, single nuclear pores are also present in this part of the nuclear envelope (Pl. IX 30, Pl. X 31, 32). When penetrating MI the omega-particle locates itself in a projection lacking chromatin elements (Pl. IX 30, Pl. X 31, 32, Pl. XI 33). In further step of penetration the bacteria dislocates into a peripheric part of MI, just under the nuclear envelope (Pl. XI 34). Simultaneously with this process a great quantity of microtubules appears in the nucleus, many of them being arranged along the bacteria (PI. XI 34, Pl. XII 35 a). Then the structure of the nuclear envelope is quickly reconstructed: perinuclear space returns to its normal dimension and the nuclear projections become smooth (Pl. XI 33, 34, Pl. XII 35 a). In some nuclei the number of symbionts attains 2–3 (Pl. XII 35 a). Around the omega-particles, situated inside the nucleus, appears an electron dense sheath, up to 30 nm thick (Pl. XII 35 a, b). This sheath is composed of fibrillar material resembling the fibrillae of chromatin elements in the *P. caudatum* MI (measuring about 10 nm in the diameter). Analogous structure has been already described occurring around jota-particles at the vegetative stage in stably infected *P. caudatum* MI (Gromov et al. 1976, Ossipov et al. 1976) and around jota-particles just after penetration into the nuclei (Ossipov and Podlipaev 1977), but never such structure has been observed in the MI of cells stably infected with omega-particles.

Not all the bacteria reach their proper locality in the MI. Some of them, together with cytoplasmic organelles and inclusions, appear in autophagic vacuoles (Pl. XIII 36, 37, Pl. XIV 43, 44) (Fig. 3); most probably they are incapable to complete the life cycle. Quite inexpectably, some cells have been observed with MA infected by omega-particles (Pl. XIV 42). In these cases characteristic structural changes of the nuclear envelope were also noted. The mode of penetration of these symbionts into the component of the nuclear apparatus unusual for them has not been elucidated.

We would like also to underline an interesting particularity in the structural changes of the MA of infected cells. Usually the MA forms a strongly developed system of thin projections, forming a kind of a basket with the MI placed inside (Pl. VIII 27 a, Pl. XI 33, Pl. XII 35 a, Pl. XIII 39, 41). The projections of MA are sometimes surrounded by flattened cysterns of endoplasmic reticulum (Pl. XIII 38, 40). According to widely accepted opinion (Raikov 1978) the changes observed in the MA express local intensification of metabolic activity, probably assuring the possibility of penetration of omega-particles into the MI.
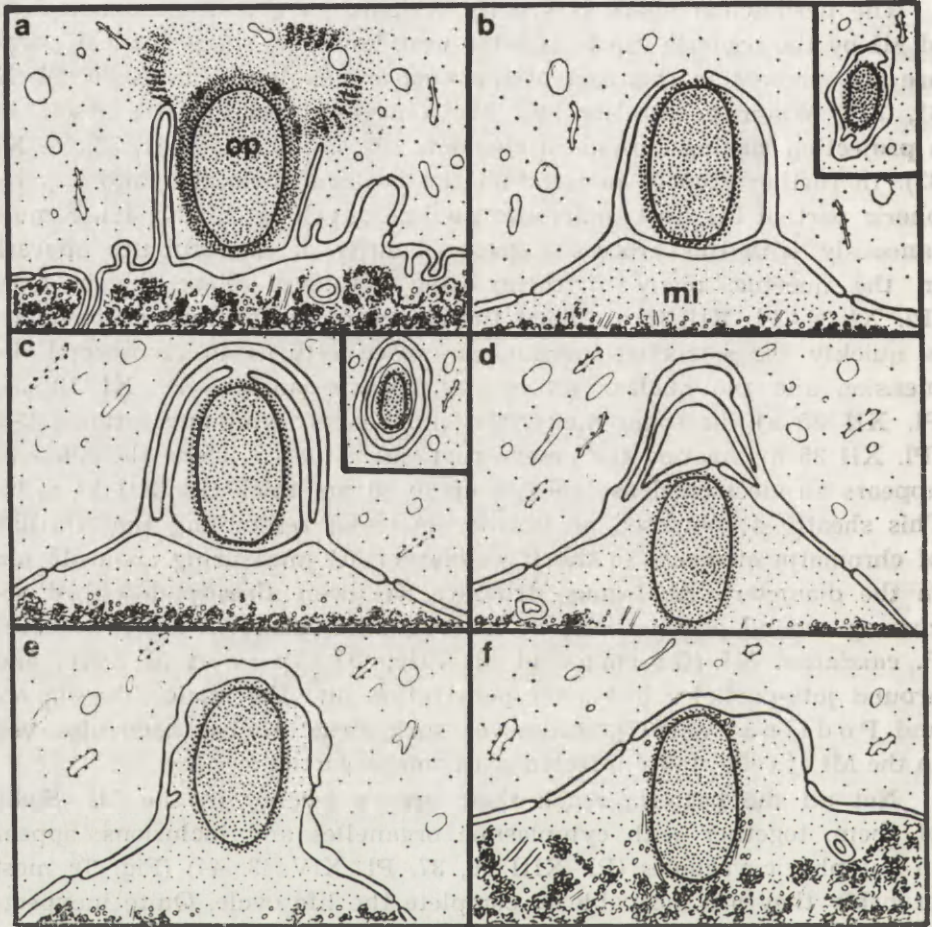
Fig. 3. Scheme of the main stages of penetration of omega- and jota-particles into the nuclei of *Paramecium caudatum*. The jota- and omega-infections are shown in one cell. Abbreviations: as in the Plates I–XIV

Discussion

The above described particularities of the infection process give the ground for the following conclusions. Infection of the MI by the bacteria, deprived of active movement capacity, is achieved due to mutual adaptations in the host-symbiont system securing transport of the symbiont from outer environment through food vacuoles and cytoplasm up to the nucleus (Fig. 3).

In our former investigations (O s s i p o v and P o d l i p a e v 1976, 1977, 1978) the ultrastructural morphology of *P. caudatum* MA, infected by another symbiotic bacteria — jota-particles, has been considered. The

results of the present paper allow to compare the penetration mechanisms of various symbionts infecting heteromorphic nuclei MA and MI. (Fig. 3, Table 1). The dynamics of infection of *P. caudatum* in both compared symbionts is not the same. Jota-particles appear in the nucleus as early

Table 1

Comparison of ultrastructural changes at early stages of infection of *Paramecium caudatum* with jota- and omega-particles

Symbiont transport stage	Ultrastructural changes and other features	jota-particles infection	omega-particles infection
Transport of the bacteria through cytoplasm	changes in ciliate cytoplasm 1 h from the beginning of infection	appearance of zones lacking endoplasmic reticulum	increase of the quantity of endoplasmic reticulum elements
	changes in spores still present in food vacuoles	maturation of spores	not observed
	mode of releasing of the bacteria from food vacuoles	deep invaginations of vacuole membrane	local bursting of the membrane (?)
	mode of isolation of the symbiont from cytoplasm	multilayer membraneous complex	sheath composed of granular and fibrillar material
	specific localization of the symbiont	macronucleus	micronucleus
Transport of bacteria through nuclear envelope	character of changes of the nuclear envelope	"sluicing" of the bacteria together with elements of membraneous complex	"sluicing" of the bacteria with membranes of nuclear envelope
	participation of nuclear microtubules in symbiont transport	not observed	probable
Errors in transport	nonspecific localization of symbionts	not observed	penetration into macronucleus sometimes observed
	falling of symbionts into autophagic vacuoles	rare	frequent

Common characters of both infections:
1. Adaptative changes in the mechanisms of food uptake and digestion under the influence of symbionts.
2. Relatively strong specificity of direct transport of the bacteria, deprived of active movement capacity.
3. Local influence of the symbionts on nuclear envelope.
4. Lack of direct contact between karyoplasm and cytoplasm during "sluicing" of symbionts

as 1 h after the beginning of infection, while omega-particles in the same time are still present in food vacuoles or in the cytoplasm.

When present in food vacuoles, jota- and omega-particles behave differently. The first of them undergo a series of changes ending in getting out of the so-called euinfective stage from the sheath of the spore-like form (O s s i p o v and P o d l i p a e v 1977). In the omega-particles such process has not been observed. Also the mode of leaving food vacuoles by these bacteria is virtually different (Table 1).

At early stages of jota-particles infection wide zones without any membranes were observed. It is supposed that the membrane material, formerly occurring in these places, takes a part in formation of the membrane complex, surrounding jota-particles Quite another picture has been observed in omega-particles infection: in the cytoplasm of paramecium, 1 h after beginning of infection, the endoplasmatic reticulum becomes much more developed than in normal cells. It is difficult to say whether it is a result of growing metabolic activity of the cell in a response to symbiont penetration or of any other cause.

The most important difference in the mechanisms of penetration by both endonuclear symbionts lays upon the following: jota-particles, after getting into *P. caudatum* cytoplasm, are surrounded by a peculiar multilayer structure — a membrane complex. We suppose that this structure performs an essential role in the transport of jota-particles to the MA and in separation of these bacteria from host cytoplasm. Such structures have not been observed in omega-particles infection of the MI. As for isolation of omega-particles from paramecium cytoplasm, this function may be performed, supposedly, by the granular sheath surrounding the symbiont. It may be seen at all stages of displacement of the bacteria, after leaving food vacuole and during their migration toward the MI (Pl. Ill 8, Pl. VII 24). This sheath has been also observed around some bacteria in autophagic vacuoles at early stages of digestion (Pl. XIV 43) as well as during penetration into the nucleus, and even just after penetration (Pl. IX 29, 30, Pl. X 31, 32, Pl. XI 33, 34, Pl. XII 35 a, b). In vegetative stages of omega-particles such sheath has not been recovered (O s s i p o v et al. 1976).

Around the omega-particles, occurring in the neighborhood of the MI, a fibrillar zone appears (Pl. Ill 9 a–c, Pl. IV 10 a, Pl. V 12 a). Its connection with cylindrical strings, laying in the cytoplasm of ciliates in a fairly great distance from the bacteria, allow to set forward a supposition about the specific role of these structures in the process of infection of the generative nucleus. It seems that formation of the sheath and of the cylindrical strings is a response reaction of the host cell against the infection. Topographical fittness of the sheath and of

the zone, in which initial stages of reconstruction of the MI envelope take place, proves that the sheath plays an inductive role in the series of changes in the nuclear envelope of MI. However, a defined localization of ultrastructural changes allows to suppose that an inductor of changes in the nuclear envelope is also acting as well as in the case of jota-particles infestation.

Inner and outer canals, surrounded by bimembranous envelope appearing in the MI, had not been observed by us in *P. caudatum* infected by jota-particles, as well as by various authors in normal generative nuclei of several species of ciliates examined in the electron microscope (E h r e t and M c A r d l e 1974, R a i k o v 1978). Intranuclear canals were described only in *Stentor coeruleus* in the moment of stretching of the vegetative nucleus during regeneration of buccal apparatus (P a u l i n and B r o o k s 1975) and in the fragments of old MA in conjugants (S k a r l a t o 1978). It is interesting that the canals are formed during the division of the nuclei in some dinoflagellates (R a i k o v 1978). Sometimes, a role of mechanic skeleton is attributed to these numerous intranuclear canals, arranged along the axis of dividing nuclei. It is possible that the above mentioned intra- and extranuclear canals, appearing for a short period of time, serve also as a scaffold, securing the possibility of penetration of larger foreign bodies (symbiotic bacteria) into the MI without any harmful effect.

It has been supposed in the study on jota-particles infection of *P. caudatum* that dislocation of the bacteria toward the MA is due to the mechanism of "relay-race" change of membrane of the host cell — of food vacuole, membrane complex and nucleus, surrounding the symbiont at subsequent stages of penetration (Fig. 3). In the omega-particles infection such change of the membrane envelopes does not occur. It is possible that the zone of fibrillar substance around the omega-particles in cytoplasm functionally plays an analogical role as the membrane complex does. In that way various structures of the host cell take a part in the mechanisms of selective penetration of the omega and jota-particles into the heteromorphic nuclei of ciliates.

The following difference between the process of infection of the MI by omega-particles and of the MA by jota-particles seems to be important. In the latter "dead-lock" way was very rare, we have never observed jota-particles in close contact with MI and only rarely they were present in autophagic vacuoles. In contrast, the omega-particles were fairly frequent in autophagic vacuoles and in some cases also in the MA. It may be supposed that getting of omega-particles into the autophagic vacuoles is connected not only with more pronounced cell reaction against the infecting agent but also with the mode of leaving

of food vacuole by these bacteria by breaking its membrane. It is possible that the zones of cytoplasm injured by hydrolytic enzymes undergo autophagy together with symbionts. While jota-particles in the moment of leaving off food vacuole form deep invaginations in its membrane, not injuring it to greater extent and thus limiting the action of digestive enzymes on cytoplasm.

Second kind of errors in the transport of symbionts — cases of MA penetration by omega-particles, may be connected simply with greater dimensions of the MA (50–70 μm) in comparison with MI (7–8 μm) and resulting greater probability of finding large nucleus by the symbiont. However, this supposition does not elucidate the fact why the omega-bacteria penetrate the MA — the habitat unusual for them. It ought to be mentioned that the reproduction and prolongation of the omega-particles life in the MA have not been observed in the electron microscope as well as in great number of investigations carried under the light microscope.

It is a seductive hypothesis to connect the differences in specific localization of both symbionts with the evolution of the system "ciliate nuclear apparatus — bacteria". Rare cases of omega-particles penetration into MA may be considered as a recapitulation proving that the initial habitat for both related species of symbionts (or for one ancestral form) was MA. Or, if we connect the development of symbiotic relationships with the appearance of nuclear dualism in ciliates, then the symbiosis of the bacteria with the MA would be regarded as primary. It must be taken into consideration that the MA of primitive heterokaryotic forms greatly resembles the nuclei of some flagellates and amoebae (R a i k o v 1978). Thus, it seems that in morpho-functional aspect the MA is closer to the ancestral type of the nuclei than the MI. At that background the jota-particles ought to be regarded as more ancient symbiont and the omega-bacteria have evolved after differentiation of a new niche — the MI. In that way, frequent finding of the omega-particles in autophagic vacuoles is a logical result of the host defense reaction against phylogenetically younger symbiont (parasite). The previous supposition about genetical connection between both species of bacteria under consideration is right even if we contribute the appearance of symbiosis relationships only to the genus *Paramecium*, i.e., to that with developed dualism of the nuclear apparatus.

Finally, the last supposition about the difference between the mechanisms of infection in both species of symbiotic bacteria. There is a supposition that the microtubules play an important role in penetration of the omega-particles through the MI envelope: the bacterium begins to dive into the nucleus in a zone of achromatic cap being sur-

rounded by the intracellular microtubules. We did not observe such kind of participation of the kinetic nuclear elements in the process of jota-particles infection.

In spite of this difference concerning, however, one of the most important stages of infection, namely the moment of penetration of the nuclear envelope, there is a substantial similarity between omega- and jota-particles (Table 1). Both types of the nuclei (MA and MI) respond in a very similar way to the infection forming a system of processes of the nuclear envelope, laying one under another. In the process of penetration the nuclear content does not come in contact with the cytoplasm. In that way the scheme of sluicing of the symbiont, reported for the jota-particles (O s s i p o v and P o d l i p a e v 1976, 1977, 1978), in basic features corresponds to that in the omega-particles (Figs. 1, 2).

Thus, the comparison of *P. caudatum* infection with omega- and jota-particles shows that the stages of intracellular transport of the symbionts, taking place in host cytoplasm, are virtually different. And, as far, the character of changes of the nuclear envelope and some other features of these processes show great similarity (Table 1).

РЕЗЮМЕ

Изучены ультраструктурные изменения клеток *Paramecium caudatum* на ранних стадиях заражения их микронуклеусов (МИ) симбиотическими бактериями — омега-частицами (ОЧ). Из культуральной среды симбионты проникают в клетку через пищеварительную вакуоль. Попадание бактерий в цитоплазму парамеции сопровождается, по-видимому, разрывом мембраны вакуоли. В цитоплазме вокруг ОЧ образуется обширная зона фибриллярного материала, который связан со своеобразными цилиндрическими тяжами диаметром около 50 нм. Последние вступают в тесный контакт с оболочкой МИ, и, по-видимому, играют определенную роль в обеспечении специфичности инфекции. Оболочка МИ в зоне контакта с ОЧ образует систему лопастей, выростов и инвагинаций. Среди них необычные для МИ инфузорий длинные внутри- и внеядерные каналы и пальцевидные выросты, содержащие микротрубочки. Выросты ядерной оболочки МИ, направленные к ОЧ, сливаются между собой и образуют вокруг бактерии замкнутую камеру, что обеспечивает предотвращение контактов кариоплазмы МИ и цитоплазмы инфузории. Возможно, что в проникновении ОЧ внутрь ядра играют роль пучки продольно ориентированных микротрубочек. Отмечены единичные случаи неспецифической локализации ОЧ в макронуклеусе (МА) инфузорий. В течение первого часа после заражения в цитоплазме инфузории наблюдаются зоны, обогащенные цитоплазматическим ретикулумом. Полученные результаты обсуждаются в сравнительном аспекте с ранее установленными особенностями проникновения йота-частиц ("шлюзование" симбионта) в МА инфузорий (Осипов и Подлипаев 1977, 1978).

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EXPLANATION OF PLATES I–XIV

1–4: Transverse sections of the omega-particles from the environment (1) and from the food vacuole of *Paramecium* (2–4) 1 — ✕ 45 000; 2,3 — ✕ 60 000; 4 — ✕ 45 000.

5: Longitudinal section of omega-particle in the food vacuole, ✕ 38 000.

6–7: *Paramecium* cytoplasm in 1 h after the beginning of infection, 6 — ✕ 22 000; 7 — ✕ 32 000.

8a: Longitudinal section of omega-particle, laying in the cytoplasm near the micronucleus. ✕ 40 000.

8b: Fragment of section 8a. ✕ 60 000.

9a–9c: Series of transverse sections of omega, laying close to micronucleus. ✕ 20 000.

10a–10d: Fragments of section 9c; 10a — ✕ 81 000; 10b–10d, 11 — ✕ 120 000.

12a: Tangential section of omega-particle, laying near micronucleus. ✕ 60 000.

12b,c: Fragments of section 12a. ✕ 100 000.

13–25: Various types of micronuclear changes of micronuclear envelope of *Paramecium*, infected by omega-particles. 13,14 — ✕ 80 000; 15 — ✕ 88 000, 16 — ✕ 85 000, 17 — ✕ 40 000, 18,19 — ✕ 35 000, 20 — ✕ 45 000, 21 — ✕ 100 000, 22 — ✕ 30 000, 23,24, 25 — ✕ 60 000.

26,27a,b,29: Longitudinal sections of omega-particles in the very moment of penetration into micronucleus. 26 — ✕ 55 000, 27a — ✕ 25 000.

27b: Fragment of section 27a. ✕ 75 000, 29 — ✕ 55 000.

28: Lightmicroscopic photograph of the micronucleus during the penetration of bacteria. ✕ 2 000. (Phot. M. S. Rautian).

30–33: Tangential sections of portions of omega within micronucleus. 30 — ✕ 50 000, 31,32 — ✕ 65 000, 33 — ✕ 50 000.

34: Longitudinal section of omega-particle, already penetrated into micronucleus. ✕ 60 000.

35a: Micronucleus with three omega-particles inside it. ✕ 30 000.

35b: Omega in the micronucleus. ✕ 60 000.

36,37,43,44: Omega-particles in the autophagic (?) vacuoles. 36 — ✕ 90 000, 37 — ✕ 25 000; 43 — ✕ 30 000, 44 — ✕ 50 000.

38–41: Ultrastructural changes of micronucleus of *Paramecium* infected by omega-particles. 38,39,40 — ✕ 40 000, 41 — ✕ 100 000.

42: The case of nonspecific localization of omega in the macronucleus ✕ 50 000.

Abbreviations: av — autophagic vacuole, cb — chromatine bodies, of the nucleus, cm — cell membrane of omega, cp — cytoplasm of *Paramecium*, cr — cysterns of endoplasmatic reticulum, cs — cylindric cords, fp — food particle (*Aerobacter aerogenes*), fv — food vacuole, fs — fibrillar zone, gs — granular envelope, ma — macronucleus, mc — mitochondria, mi — micronucleus, mt — microtubules, mv — membrane of food vacuole, nc — channel in nuclear envelope, ne — nuclear envelope, np — nuclear pore, op — omega-particle, pb — plasmatic body of omega, pl — periplasmatic layer, of the "spore", pn — perinuclear space of the nucleus, tr — tubules of the reticulum.

EXPLANATION OF PLATES I—IV

1—4: Transverse sections of the micronucleus near the food environment (1) and from the food vacuole (2). Interpretation (3)... 4 — ... 2 — × 60 000. 1 —
× 45 000.

5: Longitudinal section of cross-partition in the food vacuole. × 18 000.

6—7: Paramecium cytoplasm in 1-2 after the beginning of injection. 6 — × 32 000;
7 — × 32 000.

8a: Longitudinal section of Amoeba particle lying in the cytoplasm near the
micronucleus. × 40 000.

8b: ... ... Sb. × 60 000.

9a—9c: Series of transverse sections of mega-laying close to micronucleus. × 50 000.

10a—10b: Fragments of section 9a; 10a — × 61 000; 10b—10b, 21 — × 120 000.

12a: Tangential section of cross-particle lying near micronucleus. × 60 000.

12b—c: Fragments of section 12a. — × 100 000.

13—16: Various types of micronuclear shapes of micronuclear structure of Amoeba
lying by cross-particles. 13—14 — × 80 000; 15 — × 60 000; 16 — × 45 000.

17— × 40 000, 18,19 — × 35 000, 20 — × 45 000, 21 — × 100 000, 23 — × 70 000. 22,24
25 — × 60 000.

26,27a,b,30: Longitudinal sections of cross-particle in the cytoplasm of partition
tion 1. × ... × 60 000, 27a,b — × 60 000, 27b — × 91 000.

27b: Fragment of section 27a. × 73 000. 29 — × 65 000.

28: Ultramicroscope photograph of the micronucleus during the replication of
bacteria. × 8 000 (Phot. M. S. Bauthry)

30—33: Tangential section of section of cross-particle micronucleus. 30—32 × 90 000
31,32 — × 15 000, 33 — × 50 000

34: Longitudinal section of ... cross-particle, division in micronucleus
× 60 000.

35a: Micronucleus with three omega-particles inside Sb. × 60 000

35b: Omega in the micronucleus. × 60 000.

36,37,43,44: Omega-particles in the ctenobacteria. 36,43 (Omega) 37,44
× 25 000; 43 — × 60 000. 41 — × 20 000.

38—41: Ultrastructural changes of micronucleus of Paramecium infected by omega-
particles. 38,39,40 — × 40 000. 41 — × 180 000.

42: The case of nonspecific localization of omega in the micronucleus. × 60 000.

Abbreviations: av — ..., c — ...
cell membrane of omega, cn — cytoplasm of Paramecium, ce — centre of micronucleus
rectum, is — cytoplastic core, td — food vacuole (membrane of omega), f — food vacuole
ts — lamina pore, ma — membrane micacide, mi — micronucleus, pi — omega nuclei, nd —
micronucleus, ni — micronuclear, nu — nucleus of food, — no, is the membrane of nuclear
envelope, in — nuclei envelope micronucleus, chromid, p — omega-particle, pa — particle
of omega, pl — replication layer, of the Amoeba, xv — cytoplasmic area of the nucleus,
ts — tubule of the tubulus.

PLATE I

S. A. Podlipaev et D. V. Ossipov

auctores phot.

S. A. Podlipaev et D. V. Ossipov   auctores phot.

S. A. Podlipaev et D. V. Ossipov   auctores phot.

PLATE IV

S. A. Podlipaev et D. V. Ossipov

auctores phot.

PLATE V

S. A. Podlipaev et D. V. Ossipov

auctores phot.

PLATE VI

S. A. Podlipaev et D. V. Ossipov

auctores phot.

PLATE VII

S. A. Podlipaev et D. V. Ossipov

PLATE VIII

S. A. Podlipaev et D. V. Ossipov

auctores phot.

PLATE IX

29

30

S. A. Podlipaev et D. V. Ossipov

auctores phot.

S. A. Podlipaev et D. V. Ossipov   auctores phot.

S. A. Podlipaev et D. V. Ossipov

auctores phot.

S. A. Podlipaev et D. V Ossipov

auctores phot.

PLATE XIII

S. A. Podlipaev et D. V. Ossipov

auctores phot.

S. A. Podlipaev et D. V. Ossipov

auctores phot.

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Topography of Cortical Layer in *Amoeba proteus* as Related to the Dynamic Morphology of Moving Cell

Synopsis: Peripheral layers of different body regions of *Amoeba proteus* were investigated to reveal the distribution of the submembraneous filamentous structures (the cortex) along the axis of moving cell. The contractile layer is discontinuous — at some places the membrane is devoid of the compact and regular filamentous lining. Gaps in the contractile cortex are always found at the tips of advancing pseudopodia. Sometimes they appear also at the middle segments of lateral walls of amoeba. There is an interrelation between the topography of the filamentous layer and the dynamics of moving amoeba. It is postulated that gaps in the contractile cortex play in polytactic amoeba the same role as in monotactic forms, the role of breaches provoking the cytoplasm flow in definite directions.

Over 50 years ago M a s t (1926) localized the motive force of amoeboid movement in the peripheral cell layer: in the ectoplasmic cylinder, but he was unaware of nature and of distribution of the contractile structures. Numerous electron microscope studies (W o l p e r t et al. 1964, K o m n i c k and W o h l f a r t h - B o t t e r m a n n 1965, N a c h m i a s 1968, C o m l y 1973, H a b e r e y 1973, P o l l a r d and K o r n 1973, T a y l o r et al. 1973, R i n a l d i et al. 1975, R i n a l d i and H r e b e n d a 1975) revealed, as well in *A. proteus* as in other amoebae, in normal cells and in the glycerinated cell models, the presence of microfilaments and in most cases they indicated their peripheral distribution in the cell. K o r o h o d a and S t o c k e m (1975, 1976) found in *A. proteus* an electron dense layer beneath the cell membrane. It has been called the cell cortex and it topographically corresponds to the stratum which earlier (M a s t 1926, W o h l f a r t h - B o t t e r m a n n 1964) was referred to as the hyaline ectoplasm.

It has been demonstrated in our earlier studies (G r ę b e c k a 1978 a,b and H r e b e n d a and G r ę b e c k a 1978) that in natural mono-

tactic forms of *A. proteus* (according to the terminology of G r ę b e c k ı and G r ę b e c k a 1978) with vesicular frontal caps, and in artificial monotactic amoebae produced by oil injectıons, the stable position of the forward locomotory pole is maintained by the permanent absence oi the contractile cortex in the advancing tip of moving cell. There is a possibility that a similar mechanism may operate in polytactic amoebae, at the tips of pseudopodia during their extension phase. This hypothesis inclined us to study the topography of the contractile cortex along the axis of cell locomotion, and in particular to compare its distribution between the advancing fronts, the middle body parts, and the withdrawing regions of amoeba.

Material and Methods

Cultures of *Amoeba proteus* were maintained in Pringsheim medium and fed, twice a week, on *Colpidium* sp.

The cells manifesting usual polytactic shape were processed for electron microscopy. They were fixed with 2.5% glutaraldehyde in 0.04 M cacodylate buffer pH 7.4, at room temperature, and postfixed in buffered solution of OsO_4. The cells were then dehydrated in the increasing ethanol series and two changes of propylene oxide, and finally embedded in Epon 812. They were sectioned with an LKB ultramicrotome, stained with saturated solution of uranyl acetate followed by lead citrate, and examined with JEM 100 B electron microscope at 8C kV.

The most essential methodical problem in this study was to localize the investigated sections of amoeba periphery in respect to their former position in the migrating cell before its fixation. This objective was reached by two ways: (1) fixation of individual specimens with known motory behaviour under microscopic control in order to identify their respective functional body regions before cutting, (2) microdissection of some specimens before embedding and further preparation of isolated frontal pseudopodia or isolated uroids.

Results

The picture of the cell cortex typical for the lateral walls of the middle body regions of amoeba, is presented in Pl. I 1. The sectioning plane was perpendicular to the cell surface which allowed to demonstrate that the orientation of filaments is predominantly parallel to the cell membrane, or only slightly deviating from it. In sections obliquely inclined in respect to the cell surface (Pl. I 2), the arrangement of filaments and of their bundles appears less regular. In both cases no

filaments were found in the deeper cell layers, beneath the cortex. Such a continuous and uniform layer of fairly well orientated filaments close to the inner face of the cell membrane, is most typical of the middle regions of amoeba's trunk (between the lateral pseudopodia) and of the proximal parts of large old pseudopodia.

However, the areas with orderly arranged filaments adjacent to the cell membrane sometimes appear alternately with areas where the filamentous layer is disengaged from the membrane and loses the regularity of its orientation (Pl. I 3). Pictures in the Pl. II 4, 5 demonstrate that the uroid is characterized by the abundance of filamentous structures disposed in a chaotic manner at different cytoplasm layers. Remnants of the former cortical pattern may be exhibited by residues of old contracting pseudopodia which have been incorporated into the uroidal zone. The residual pseudopodium shown in the Pl. II 5 has a prominent peripheral layer composed of very densely packed filamentous material, which is distinctly separated from the more axial regions. The axial part is also filled with filaments, with no other visible cytoplasmic components, but they are much less abundant than in the cortical zone.

In some segments of the middle body regions of amoeba the filamentous material may manifest uneven density and irregular distribution beneath the cell membrane. In the area shown in the Pl. Ill 6 the bundles of filaments form discontinuous bands which run parallel to the cell surface at some distance from the membrane. Between these bands and the cell surface some filaments are probably attached to the membrane.

The most pronounced irregularities in conformation of the cortical layer, its dissociation from the cell membrane and its discontinuity, appear at the places which may be identified as areas of origin of new pseudopodia. Numerous small new hyaline pseudopodia formed after contraction of the frontal region of an amoeba exposed to a mechanical shock are seen in the Pl. Ill 7. One of them is shown in higher magnification in the Pl. IV 8. The interior of new pseudopodia appears almost electron empty. Only small vacuoles and vesicles may often be seen in their inner layers, but filaments are either absent or very scarce beneath the cell membrane. They are found at the basis of new pseudopodia (and sometimes at the basis of frontal caps of old advancing pseudopodia) in the form of well organized bands of filaments transversally intersecting the axial region of the pseudopodial basis. In the Pl. IV 8 one can see the strictly perpendicular orientation of these filamentous bands in respect to the pseudopodial axis and its continuity with the regular submembraneous cortex on both sides of the pseudo-

podium. Such pictures may suggest that the bands of filaments projecting toward the axial region of a new pseudopodium at its basis, are remnants of the typical cortical layer which is disrupted by the cytoplasm streaming at an early stage of pseudopodium development.

All the tips of large and well developed advancing pseudopodia studied in the present research were in some extent similar to those of new hyaline pseudopodia. The typical regular cortex could be found at some distance behind the advancing front. At the same distance some filamentous material was often found also in the axial region, either in disorganized pattern or in the form of transversal bands. But the typical well developed cortex was never detected in the tip itself, on the territory corresponding to the frontal cap. An example of typical picture presented by this most frontal area is shown in the Pl. IV 9. Only very few filaments may be seen beneath the frontal cell membrane, instead of the distinct and well organized contractile layer which was found in the lateral walls of the same individual. It should be emphasized again that the pictures demonstrating the absence of a distinct cortex in the tips of advancing pseudopodia and its presence in the lateral walls of amoeba were obtained from the same specimens cut after the fixation or even from different parts of the same sections (cf. Pl. III 7 and IV 8).

The Pl. III 7 demonstrates that the continuous cortex is present under membrane of the former anterior region of amoeba which was induced to retract under the influence of a mechanical shock.

Discussion

The present results are fully consistent with those of K o r o h o d a and S t o c k e m (1975) and of R i n a l d i and H r e b e n d a (1975) as to the clearly peripheral distribution of the filamentous material and its localization just beneath the cell membrane. The extent of the filamentous layer observed in the present study fits in well with the position and thickness of the lateral "hyaline ectoplasm" as defined by W o h l f a r t h - B o t t e r m a n n (1964).

In the paper of R i n a l d i and H r e b e n d a (1975) concerning the arrangement of thin and thick filaments in *A. proteus,* the electron microscope pictures were presented in which the filaments locally formed patterns similar to the pattern known in the muscle fibres. Both types of filaments were running parallel to the cell surface, and some thin filaments seemed to be attached to the membrane. In small pseudopodia and in the frontal parts of larger ones the filaments were not

observed. The electron microscope pictures obtained in the present study corroborate these earlier conclusions, in particular as to the predominantly parallel arrangement of filaments in respect to the cell surface in lateral walls of amoeba.

However, the main objective of the present research was not to investigate the internal structure of the cortex, but its topographic distribution in a normally migrating cell of amoeba. It has been shown in our precedent paper (H r e b e n d a a n d G r ę b e c k a 1978) that in monotactic forms of *A. proteus* the vesicular frontal cap (or the artificial oil cap) locally isolates the cell membrane from the contractile material. There is no filamentous cortex between the membrane-like cap envelope (or oil) and the cell membrane. The conclusion has been drawn that this local discontinuity in the cortical layer (i.e., the local incapability of any countercontraction) accounts for the stable position of front in such amoebae "attracting the cytoplasm streaming and determinating the monotactic type of body shape and of locomotion". Only a spontaneous (G r ę b e c k a 1978 a) or experimentally induced (G r ę b e c k a 1978 b) penetration of endoplasm between the cap and the frontal outer membrane may reconstitute the cortical continuity, and then, promote changes in cell motory polarity leading to the polytactic mode of locomotion. Therefore, the question arose in what extent a similar explanation might apply to the orientation of streaming and locomotion in polytactic amoebae. It should be, of course, taken into account that in polytactic amoebae lacking stable frontal caps, rather numerous periodically appearing and disappearing openings in the filamentous cortex (instead of a permanent single breach) are expected.

The electron micrographs presented in this study permit to conclude that the filamentous cortical layer is not a perfectly continuous structure enveloping the whole body in a uniform manner. Separate investigation of definite body regions demonstrated the existence of some differences in distribution of cortical material in respect to the dynamic morphology of migrating cell. In the uroidal zone of amoeba a thick and fairly compact system of filaments, rather chaotic in orientation, is usually observed. In the lateral walls of middle body regions the cortex is most often seen as strictly peripheral uniform layer of filaments parallel to the cell surface and/or in the form of bundles deviating from the membrane. Occasionally, some areas may be found where the filamentous layer seems to be dissociated from the membrane. Any regular filamentous layer was never seen in the present material, beneath the cell membrane of the most frontal zones of advancing pseudopodia. This defficiency of cortical layer on the territory of active frontal caps may be observed as well on some electronograms published erlier by

Rinaldi and Hrebenda (1975 and Grębecka and Hrebenda (1979).

Results of the present study incline to suppose that the filamentous cortical layer in polytactic *Amoeba proteus* is discontinuous: (1) at the tips of active advancing pseudopodia, in the areas of their frontal caps, (2) around the protuberances formed by new developing pseudopodia, and (3) at some other places scattered along the body walls which may be considered as spots predestinated to produce pseudopodia. In these three localizations the cortex is not visible in the present electronograms, although it is well fixed in other sections obtained from the same cell, or even in other parts of the same sections.

The areas devoid of organized filamentous material may be presumed to present lesser elastic resistance to passive dilatation and are certainly incapable to countercontract, what enables the endoplasm influx, the development and the further growth of psudopodium. This argumentation is supported by recent light microscopic observations of Kalisz-Nowak (1978) indicating that some changes in the cortex (development of an optically empty spot) precede the initiation of a new pseudopodium. The existence of gaps in the contractile cortical layer is also consistent with the generally known phenomenon of instantaneous massive protrusion of numerous small pseudopodia producing a rosette-like body shape of amoebae in which the general contraction has been induced by a shock stimulation.

It should be noticed, however, that the present results and interpretations seem inconsistent with the high electron density of hyaline caps demonstrated by Korohoda and Stockem (1975) in amoebae which were not allowed to locomote freely but were deformed by a pretreatment with ethanol. The persisting uncertainty about some properties of frontal caps of polytactic amoebae should be probably resolved not in the fixed material but by experimentation on living and actively moving cells.

Another question raised by the present results concerns the significance of transversal filamentous bands which intersect the basis of new pseudopodia and sometimes are seen behind the frontal caps of well developed advancing pseudopodia. Are they remnants of a transversal filamentous layer periodically disrupted by the endoplasmic streaming? That could explain the movement by spurts manifested by the pseudopodial tips and the "plasmagel sheet" nature and behaviour, which has been originally described by Mast (1926) and re-studied more recently by film analysis (Rinaldi 1964 a, b). The supposition that the "plasmagel sheet" might by composed of a filamentous layer alternately regenerating and bursting behind the frontal cap of advancing pseudo-

podium fits in well with the birefringence pattern of "old plasmagel sheet" which indicates "an orientation of linear elements perpendicular to the pseudopodial axis" (A l l e n 1973).

The electron microscope pictures of residual contracting pseudopodia incorporated into the uroidal zone, and of the former front of amoeba contracted in response to a mechanical shock, allow to suppose that probably the transition of pseudopodium from the expanding to the contracting state may be correlated with a stable reconstruction of cortex around its tip.

In general, the data exposed in this report confirm again the peripheral distribution of the organized contractile material in amoeba, and consequently: the peripheral origin of the motive force and the squeezing mechanism of the endoplasm movement, as it was postulated by M a s t (1926). Among several slightly different interpretations of the original ideas of Mast proposed by his followers, the concept of general contraction of the whole peripheral layer, suggested first by M a r s l a n d (1964) and recently reiterated by G r ę b e c k i (1979), has been choosen as a plausible explanation of the obtained results. According to it, the presence of breaches in the contractile cortical layer and their topography in respect to the dynamic morphology of amoeba, as described in this study, may explain the initiation of the endoplasm flow in a given direction, its continuation, and the extension of pseudopodium.

RÉSUMÉ

Les couches périphériques des différentes régions de la céllule de l'*Amoeba proteus* étaient examinées pour mettre en évidence la répartitiont des structures filamentaires (le cortex) le long de l'axe de locomotion. L'ènveloppe contractile formée par le cortex n'est pas continue — des endroits existen où la face intérieure de la membrane céllulaire n'ést pas couverte d'une couche compacte et régulière des microfilaments. On trouve toujours de telles lacunes aux extrémités des pseudopodes en extension. Parfois elles apparaissent aussi dans les parois latérales de l'amibe. La topographie de la périphérique couche contractile s'avère liée à la dynamique de locomotion de la céllule. On peut conclûre que les lacunes formées dans le cortex contractile jouent chez les amibes polytactiques le même rôle que chez les monotactiques, le rôle des orifices qui dirigent le courant cytoplasmique dans des directions définies.

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Note added in proof:

Recently appeared important study of J. Wehland, K. Weber, W. Gawlitta and W. Stockem: Effects of the actin binding protein DNAse I on cytoplasmic streaming and ultrastructure of *Amoeba proteus*. An attempt to explain amoeboid movement. Cell and Tissue Research, 199, 353–372. These authors also present pictures proving the discontinuity of cortex or of its dissociation from the cell membrane in the extending regions of amoeba, and draw the same conclusions concerning their motory role as we suggested here and in our earlier reports. Unfortunately, the results and concepts of Wehland et al. dated on April 1979 were unknown to us when we were preparing the manuscript of the present paper in October 1978.

EXPLANATION OF PLATES I–IV

1: Periphery of middle body region of *A. proteus*. Section perpendicular to the cell membrane. Note the layer of filaments (F) lying close beneath the membrane and parallel to its surface. In the deeper cell layers the filaments are not visible

2: Periphery of middle body region, cut obliquely in respect to the cell surface. Note the filaments (F) and their bundles (FB)

3: Section of cell periphery from the posterior body region of amoeba. The layer of filaments, well orientated and closely related to the cell surface, on the left and right sides of the picture, is dissociated from the membrane and loses its regularity in the central area (between arrows)

4: Fragment of the uroid of *A. proteus*. Note the abundance of filaments (F), their chaotic distribution and random orientation

5: Another fragment of uroid, with a residue of former contracting pseudopodium. Note the sharp difference in the density of filaments between the peripheral remnants of cortex (Cx) and the axial parts of pseudopodium (A)

6: Example of irregular cortical layer from the middle body regions. The filaments form a band (FB) running parallel to the cell surface but distincly separated from the membrane. Only scarce filaments (F) are seen between the membrane and the band, which disappears in the left part of the picture

7: Frontal zone of amoeba after a general contraction induced by mechanical shock. Note the presence of continuous cortical layer (Cx) under the cell membrane, and numerous small hyaline pseudopodia

8: Proximal part of a small hyaline pseudopodium produced in the frontal region of amoeba after its contraction induced by mechanical shock. The picture is a higher magnification of fragment of the pseudopodium shown in Pl. Ill 7 by arrow. Note the absence of filaments inside the pseudopodium, the regular perpendicular bands of filaments (FB) intersecting its basis and their connection with the regular cortex (Cx) on both sides

9: Tip of a frontal advancing pseudopodium. Note the scarcity and irregularity of filaments failing to form any organized cortical layer under the cell membrane

PLATE I

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PLATE II

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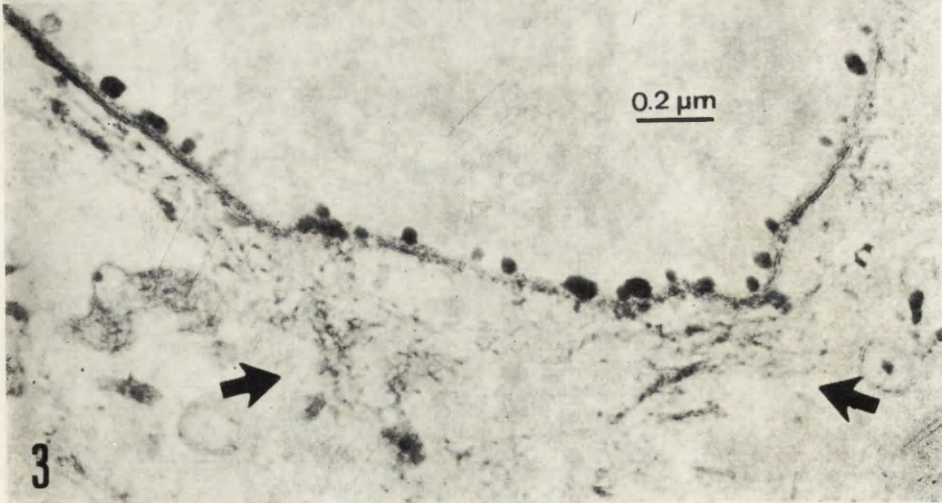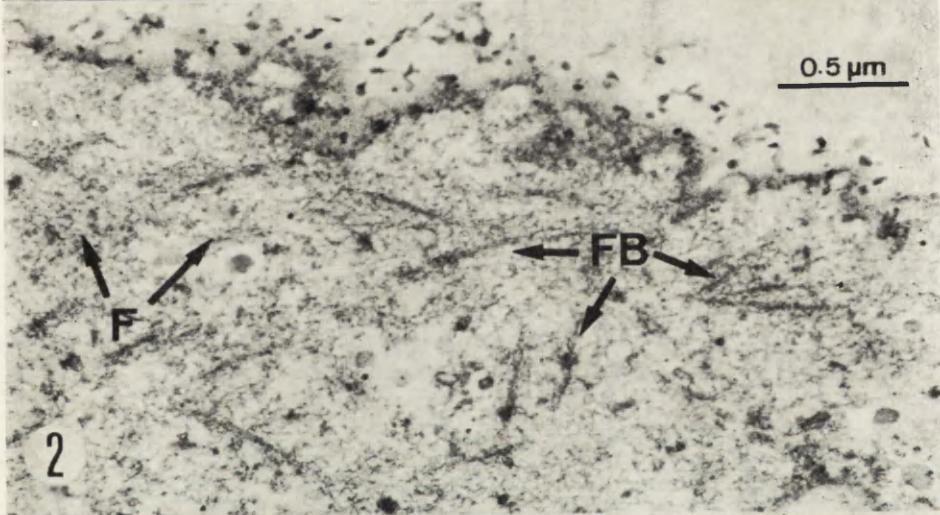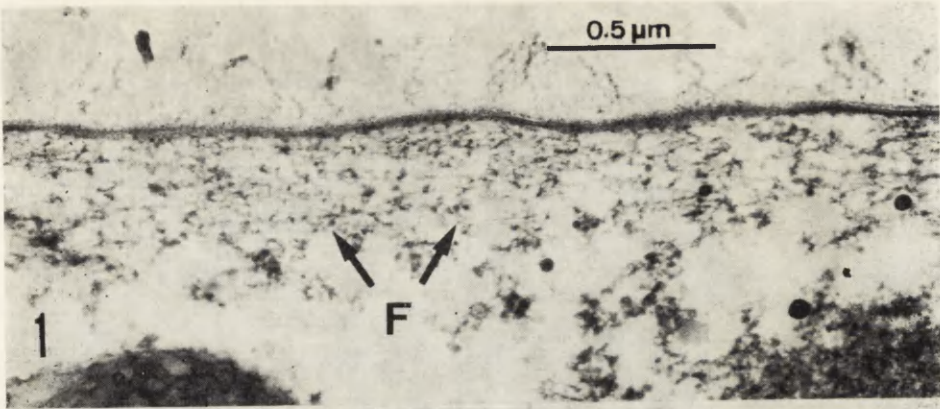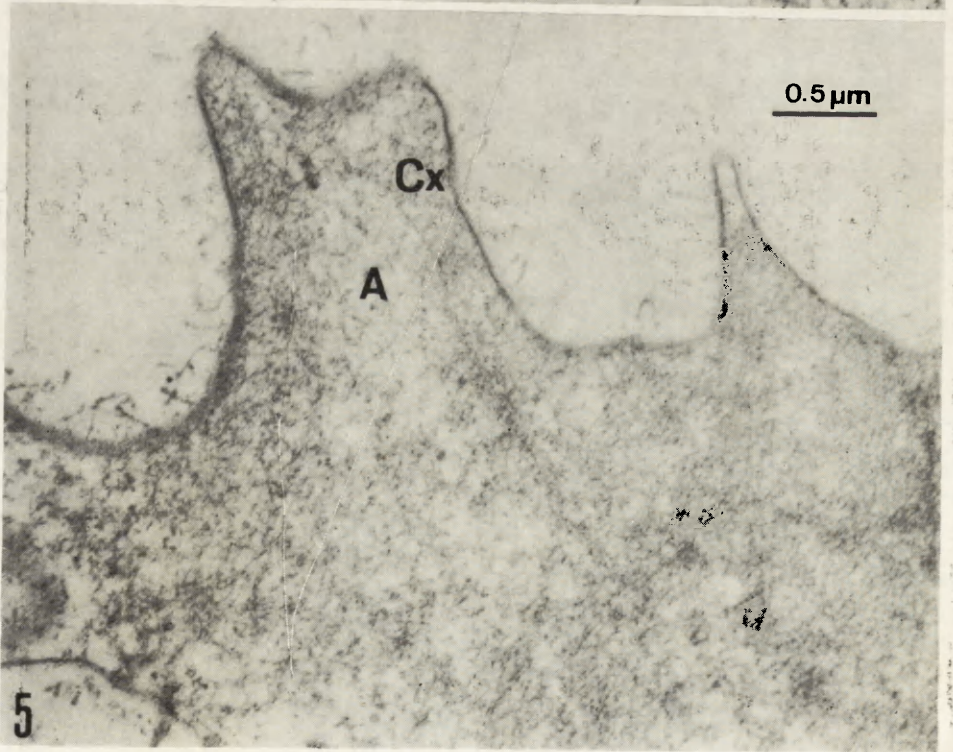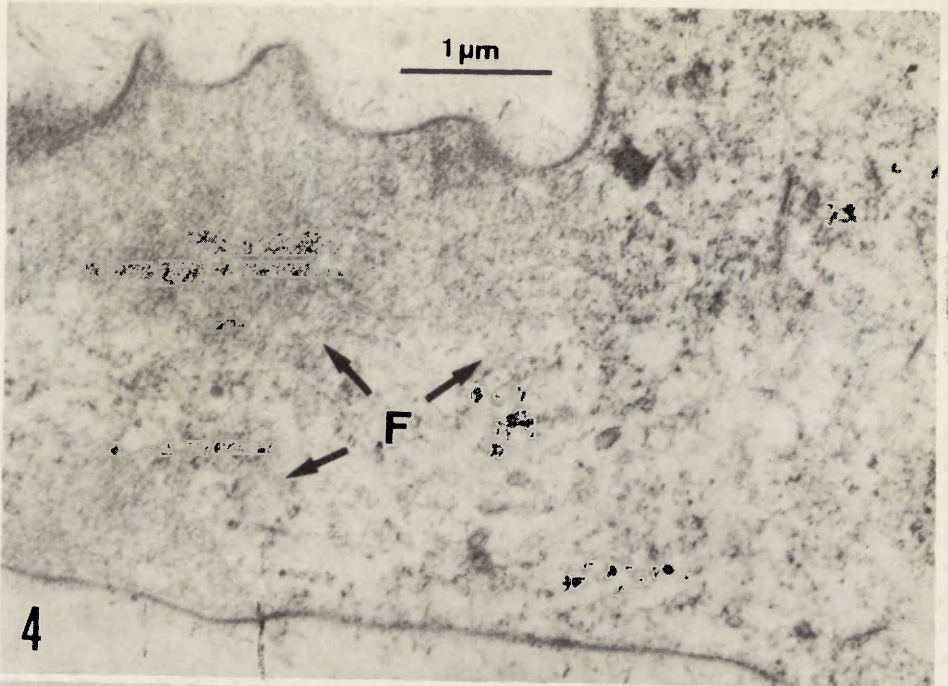
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PLATE IV

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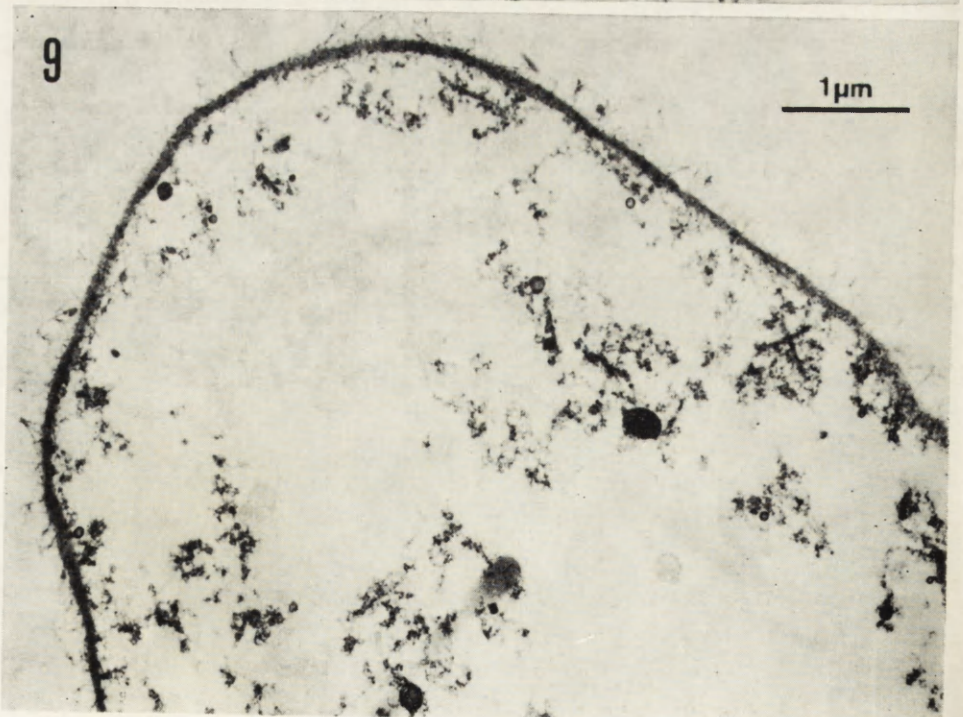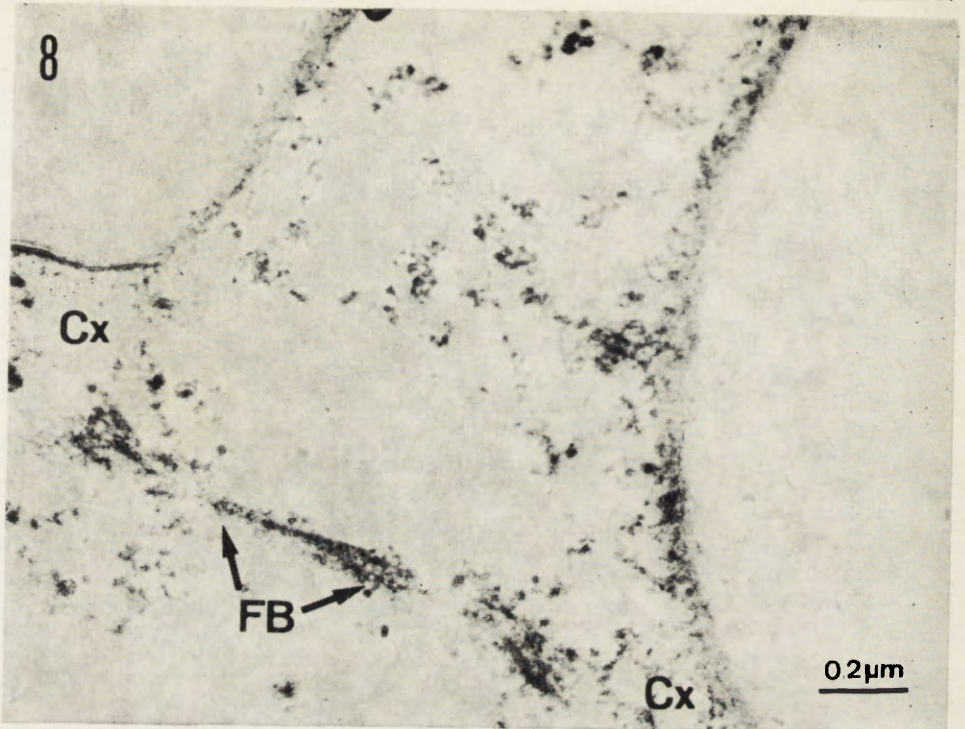
György CSABA and László UBORNYÁK

Quantitative Observations on Triiodothyronine and Histamine Binding in *Tetrahymena*

Synopsis. The unicellular *Tetrahymena pyriformis* possesses structures capable of binding histamine and triiodothyronine. Support for the specificity of binding has been emerging from depression of binding of label and displacement of bound label by non-labeled hormone given before and after treatment with labeled hormone, respectively.

Although unicellular organisms are by nature devoid of a hormonal regulation, they possess certain hormone-like material which also occurs in higher organisms (B l u m 1967, H i l l 1972, J a n a k i v e d y et al. 1976) and are able to respond to others which are non-physiological at the unicellular level (L e g r o s et al. 1975). Such responses are as a rule specific. Serotonine and histamine enhance the phagocytotic activity of *Tetrahymena* (C s a b a and L a n t o s 1973, 1977), and the ciliate is able to distinguish these amines from chemically related compounds or antagonists (C s a b a and L a n t o s 1975 a). Adrenalin (C s a b a and L a n t o s 1976) and insulin (C s a b a and L a n t o s 1975 b) stimulate the glucose metabolism in *Tetrahymena*, although not in the same manner, and insulin and iodine hormones (T_3, T_4) become specifically bound by certain membrane structures of the ciliate. Several hormones occurring exclusively in vertebrates stimulate rise of the cAMP level in *Tetrahymena* (C s a b a and N a g y 1976).

It follows from the foregoing observations that the *Tetrahymena* does posses membrane structures equivalent to the receptors of higher animals. These structures although obviously nonspecific — enter into a reaction with ligands (hormones) under artificial conditions.

Quantitative observations supporting the specificity of hormone binding by *Tetrahymena* are reported in this paper.

Material and Methods

Study of Triiodothyronine Binding

(a) Samples of 10^6 cells from 3-day cultures of the *Tetrahymena pyriformis* GL strain were suspended in 6 ml Losina-Losinsky (1931) solution, and 1μCi ^{125}I-triiodothyronine (T₃; Amersham-U. K., spec. act. 50 Ci (g) was added to each sample for an incubation period of 45 min.

(b) The ciliates were kept in a 2×10^{-7} M solution of non-labeled T₃ for 30 min, thereafter the unbound T₃ was removed by several centrifugations and washings (in Losina solution). Subsequently radioisotope treatment was carried out as described in paragraph A.

(c) Cells treated as in paragraph A were exposed — after repeated centrifugations and washings — to 2×10^{-7} M non-labeled T₃ in Losina solution for 15 min.

After the procedures described — in each group — the ciliates were killed by addition of 0.1 ml formaline to the system, and were centrifuged at 5000 r.p.m. for 5 min.

Samples for radioisotope assay (Packard-Tricarb scintillation counter) were obtained from the supernatants in each system as follows: (1) a sample from the incubating medium not containing the cells, (2) another sample was taken from the supernatant after killing ciliates and centrifuging out the cells; the measured differences between the activities of the two samples reflect the amount of the labeled T₃ bound by the cells. In the system C, three samples were taken for determining the amount of the specifically bound isotope displaced by the non-labeled T₃.

Study of Histamine Binding

Following groups of ciliates have been studied:

(a) Samples of 10^6 cells from 3-day cultures of *Tetrahymena pyriformis* GL strain were suspended in 6 ml Losina-Losinsky solution, and 4 μCi ^{3}H-histamine-dihydrochloride (Amersham, U.K., spec. act. 10.9 Ci) was added to each sample for an incubation period of 45 min.

((b) Treatment as in group A had been preceded by 30-min exposure to 10^{-8} M non-labeled histamine.

(c) Treatment as in group A was followed by 15-min exposure to 10^{-6} M non-labeled histamine.

The further procedures (centrifugation, sampling, radioisotope assay, etc.) were the same as in the first experiment.

In each group 2×17 samples were evaluated with the scintillation counter.

Results

The experimental results are shown in Table 1. Pretreatment caused a significant decrease of T₃-binding, while post-treatment led to a marked displacement of bound labeled hormone. The measured values —

Table 1

Binding of triiodothyronine and histamine by *Tetrahymena* in different groups
(further explanations in chapter Materials and Methods)

^{125}I-triiodothyronine		^{3}H-Histamine	
Cells	Cpm/10^5 cells	Cells	Cpm/10^5 cells
Control	271.11 ± 44	Control	4287.17 ± 679
Pretreated with non-labeled hormone	$167.8 \pm 29^+$	Pretreated with non-labeled hormone	$3035.69 \pm 510^{++}$
Post-treated with non-labeled hormone	$194.25 \pm 42^+$	Post-treated by non-labeled hormone	$3645.97 \pm 565_{\circ}^+$

Related to control: $p < 0.01 = +$, $p < 0.001 = ++$.
Related to pretreated: $p < 0.02 = 0$.

neither following the pretreatment nor in the case of the post-treatment — did not differ significantly.

The binding of labeled histamine, too, was significantly decreased by pre-treatment, and a substantial part of bound labeled histamine was displaced by non-labeled hormone as a result of post-treatment. The measured values differed significantly between the experimental groups (pre- and post-treatments) as well as between that and the control groups.

Discussion

The surface of the cell membrane is covered by an external coat (surface coat, glycocalix). The integral membrane proteins emerging from the cell membrane are complexed with the oligo- and polysaccharide components of the glycocalix delivering specific membrane patterns, first of all specific cell marker and receptor structures. The latter structures serve as sites of linkage for the ligands. Independently of this interaction, the plasticity and special electric charges of the external coat make possible the adsorption of certain molecules seemingly like a true linkage. The nature of this binding can be clarified by two different experimental ways: (1) first one treats the cells with the non-labeled (cold) ligand — for binding the free receptors and thereafter adds the labeled substance or (2) after adding the labeled substance one treats the cells with the non-labeled one, much in excess, to displace the labeled molecules from the binding sites (B l e c h e r 1976, J a n a-k i v e d y et al. 1976). In both cases the effects showed similar tendency, namely: (a) when the receptors are already bound by non-labeled mole-

cules the labeled ones can bind to a lesser degree and (b) the measurable amount of the labeled substance decreases proportionally to its displaced quantity. The latter method is the demonstration of specificity, since only the specifically bound molecules can be displaced on the membrane receptor, the adsorbed ones not.

Mathematical–statistical evaluation has shown that significantly less labeled T_3 and histamine was bound by *Tetrahymena*, if non-labeled hormone had been added to the system before or after exposure to labeled hormone.

If non-labeled hormone was used for pretreatment, subsequent exposure to the labeled hormone resulted in displacement of the former one by the latter one from the receptors. As labeled hormone was added to the experimental systems at a much lower concentration than the non-labeled one, and displacement has been known to be time-dependent, the period of pretreatment was kept shorter than that of exposure to label. The quantitative loss of label nevertheless differed significantly between the pre- and after-treated systems. This observation and the fact that full displacement of labeled by non-labeled molecules and vice versa could never be achieved, cannot be explained by the time factor alone and requires further study.

The T_3 and histamine molecules bound by the membrane become incorporated by the *Tetrahymena* cell via endocytosis (C s a b a et al. 1977, H i l l 1972). Assays of bound label included, as a matter of fact, both membrane-bound and incorporated labeled hormone. Pretreatment with non-labeled hormone *ab ovo* resulted in binding of smaller amount of label than normally, while on after-treatment only the membrane-bound part of label could be displaced by non-labeled hormone, the incorporated part not. This can account for the differences found between binding of label on pre- and post-incubation with non-labeled hormone, and for the incompleteness of displacement as well.

ZUSAMMENFASSUNG

Der einzelliger *Tetrahymena pyriformis* hat die Membranen-strukturen, welche können das Histamin und das Triiodothyronine binden. Die Spezifizität der Bindung beweist die Bindungverringerung Effekt der vorausgegangenen Behandlung mit nichtmarkiertem Hormon, und die Nachbehandlung, die ergeben einen Austasch der Bindung des markierten Hormons.

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Jerzy J. LIPA and Irma V. ISSI

Histological Observations on Transovarial Transmission of *Nosema heliothidis* L. et S. (*Microsporidia*) in *Laphygma exigua* Hbn. (*Lepidoptera, Noctuidae*)

Synopsis. The moths of beet armyworm (*Laphygma exigua* Hbn.) collected in field in Tadzhykistan (USSR) were infected by a microsporidian identified as *Nosema heliothidis* L. et S. The parasite caused a general infection and its spores measured 3.2–4.9 × 2.1–3.0 µm. The spores and developmental stages of the parasite were observed in oogonia, oocytes, trophocytes and in follicular epithelium of ovarioles.

A beet armyworm (*Spodoptera exigua* Hbn.), also known as caradrine, is a widely distributed noctuid recognized as a serious pest of various plants in different regions of the world. Therefore, the knowledge of pathogens of this pest is important for development of biological control methods.

Several pathogens are known from *S. exigua* including viruses, fungi and bacteria. As far as protozoans are concerned E v l a c h o v a and Š v e c o v a (1965) recorded in *Spodoptera* (= *Laphygma*) *exigua* two microsporidians: *Nosema* sp. and *Thelohania* sp.

In this paper we report on a development of *Nosema* sp. in ovaries of *S. exigua*.

Material and Methods

The female moths of *Spodoptera exigua* were collected in summer of 1967 in the Tadzykistan (Tadzhyk Soviet Socialistic Republik). They were dissected, their gonads were fixed in Bouin's fluid, routinely embedded into paraffin, then cut and stained with Heidenhain's hematoxylin and eosin, mounted in Euparal, and viewed and photographed with a Zeiss Jena photomicroscope.

Results

Taxonomic Position of Pathogen

The microsporidian involved belongs to the genus *Nosema* what is indicated by the fact that spores and developmental stages are distributed singly within the host's tissues and do not occur in groups or pansporoblasts.

E v l a c h o v a and Š v e c o v a (1965) recorded in *S. exigua* two protozoans *Nosema* sp. and *Thelohania* sp. but without giving information on spore dimensions and tissues attacked.

W e i s e r (1960) from a closely related species *Spodoptera* (= *Laphygma*) *frugiperda* (Smith) described *Nosema laphygmae* infecting fat body of its host and having spores 4.0–5.0 × 1.5 × 2.0 μm in size and macrospores 8.0 by 2.5 um.

Since *Nosema* sp. observed by us in *S. exigua* has no macrospores and causes the general infection it cannot by identified with *Nosema laphygmae* Weiser.

The spore dimensions of *Nosema* sp. and pathological features it causes in *S. exigua* indicate its identity with *Nosema heliothidis* L. et S. a well known pathogen of *Heliothis* spp. (K r a m e r 1959, L i p a 1968 a). The fixed and stained spores of *N. heliothidis* are 2.8–5.0 × 2.0–3.0 um (L i p a 1968 a) while *Nosema* sp. have spores 3.2–4.9 × 2.1–3.0 um. The detailed comparison of spore length of both microsporidians is given in Table 1.

Histological Studies

Ovaries of *Spodoptera exigua*, like of other *Lepidoptera*, consist polytrophic ovarioles in which trophocytes accompany each oocyte and are enclosed within the follicle.

In Plate I 1 we see a general view of a section through an ovariole well showing oocytes, trophocytes and follicular epithelium. At the

Table 1

Frequency distribution of length of two samples of 50 spores of *Nosema heliothidis* L. et S. and *Nosema* sp.

Microsporidian	Dimensionable groups [μm]				
	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0
Nosema heliothidis L. et S.	1	12	23	11	3
Nosema sp.		7	31	11	1

terminal end of the ovariole oogonia are seen. The infected part is indicated by a black rectangle.

In a consecutive Pl. I 2, 3 one can see enlarged portions of follicular epithelium with several schizonts and spores of *N. heliothidis*. In the primary oocytes enclosed by follicular epithelium several schizonts are readily seen (Pl. I 2).

It is interesting to notice that the follicular epithelium infected by *N. heliothidis* is multilayered due to proliferation. The normal follicular epithelium is unilayer.

In a series of Pl. II 4, 5 and 6 it is even more clearly seen that especially heavily are infected trophocytes. The spores of *N. heliothidis* are located in epithelial cells in the trophocytes and also in protuberances made by trophocytes into the oocytes (Pl. II 7).

Discussion

K r a m e r (1959) and L i p a (1968 a, b) have noted the transovarial transmission of *N. heliothidis* in *Heliothis zea* (Boddie). This fact has been histologically confirmed by B r o o k s (1968) in *H. zea*.

In this paper we present a set of photographs clearly showing the development of *N. heliothidis* in ovaries of *Laphygma exigua* and the mechanism by which spores are transferred to eggs.

The microsporidian infects most heavily the trophocytes and follicular epithelium in ovarioles. When these cells burst due to hypertrophy the spores fall into oocytes directly or through nutritive spores. Consequently, the spores or other developmental stages may be seen within the eggs, developing embryos or in hatching larvae.

The microsporidian infects most heavily the trophocytes and folliferation of follicular epithelium (Pl. I 2, 3). It is not clear whether multilayer epithelium creates better or worse conditions for the development of oocytes and, consequently, the eggs.

RÉSUMÉ

Les femelles de *Laphygma exigua* Hbn. récoltés dans des conditions naturelles à Tadjikistan (URSS) étaient infecteés par une microsporidie identifiée comme *Nosema haliothidis* L. et S. Le parasite provoquait une infection générale. Les dimensions des spores se situaient entre 3.2–4.9 × 2.1–3.0 μm. L'étude histologique des ovarioles a prouvé la présence des spores et d'autres stades du parasite dans les oogonies, les oocytes, les trophocytes et dans l'epithèle folliculaire.

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EXPLANATION OF PLATES I–II

Nosema heliothidis L. et S. in ovarioles of female moths of *Laphygma exigua* Hbn

1: A general view on a section through an ovariole with indicated infected region
2,3: Spores (S) and other developmental stages (Sc) seen in follicular epithelium
and trophocytes
4: A section through an ovariole with indicated by arrow infected protuberances
of trophocytes
5,6: Enlarged protuberances and oocytes infected by the parasite: S-spores

PLATE I

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auctores phot.

PLATE II
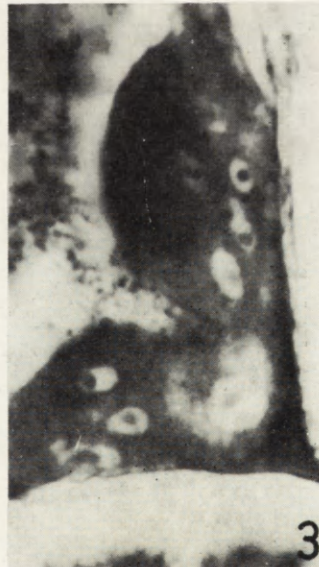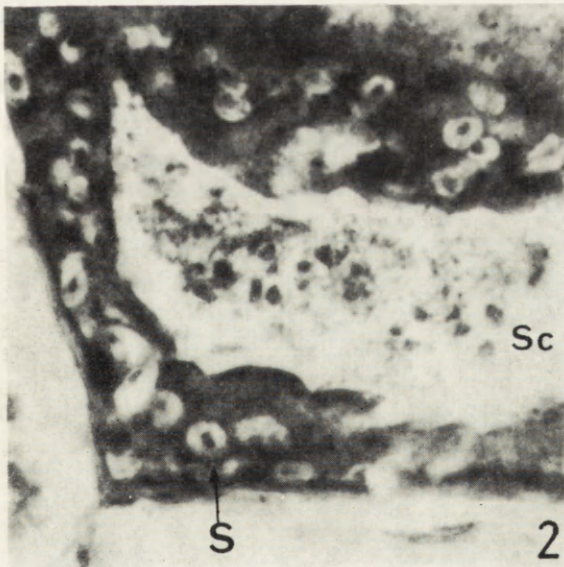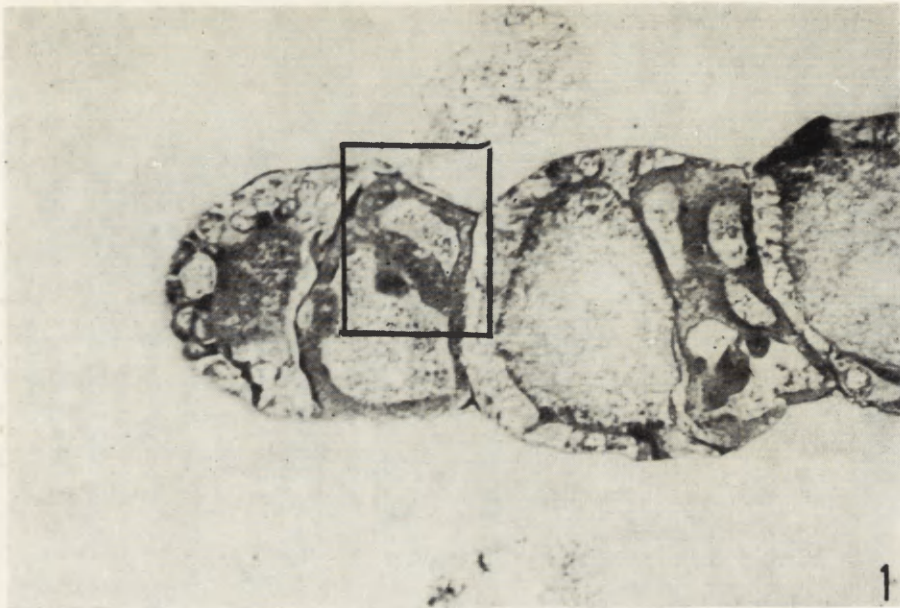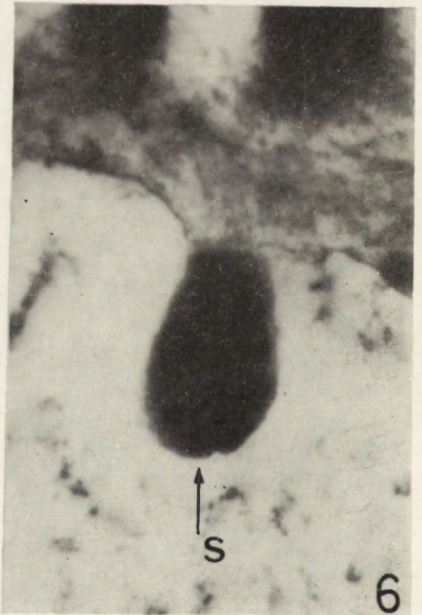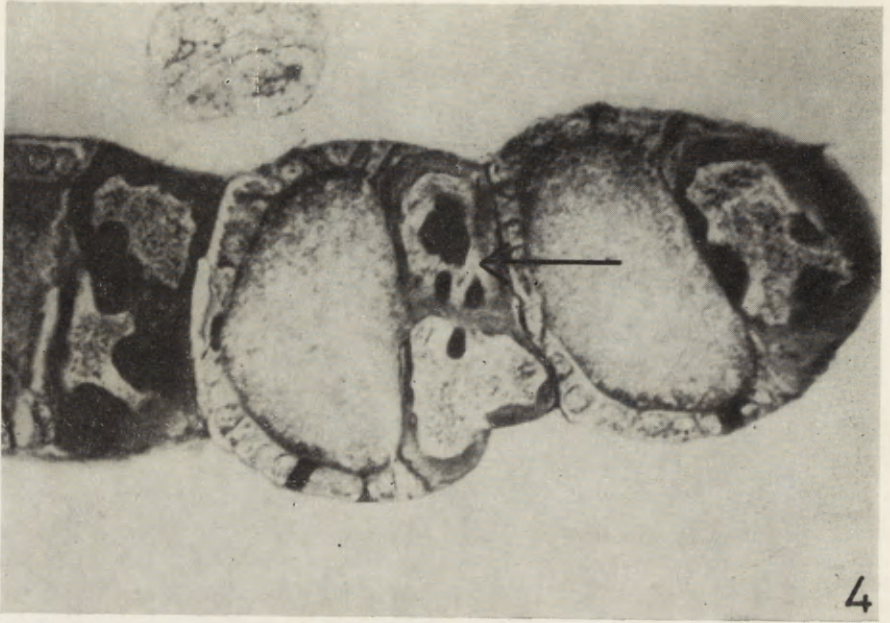
J. J. Lipa et I. V. Issi

auctores phot.

First Announcement

Sixth International Congress of Protozoology is organized by the M. Nencki Institute of Experimental Biology in Warsaw, the Committee on Cell Biology of Polish Academy of Sciences (National Group of ECBO: a Federation of European Societies for Cell Biology) and the Protozoological Section of the Polish Zoological Society. It wil be held in Warsaw on 5–11 July, 1981.

The Congress is open for Protozoologists from all countries and is not limited to members of organized protozoological societies.

The Programme will include: Invited Lectures from main fields of protozoology, Symposia, Contributed Paper Sessions and Poster Sessions.

The Symposia and Contributed Paper Sessions will be grouped into six sections:

> Systematics and phylogeny of Protozoa
> Genetics and morphogenesis
> Development and life cycles
> Physiology, biochemistry and immunology
> Motility and behaviour
> Ecology of free-living and parasitic Protozoa

Scientists interested in participating in the Congress should write, before 1 March 1980, to:

> Dr. Stanisław L. Kazubski
> Secretary General
> VI International Congress of Protozoology
> M. Nencki Institute of Experimental Biology
> 3. Pasteur str., 02-093 Warsaw, Poland

They will then receive the Second Announcement, registration forms, details about housing, as well as information about social activities, entertainment and tours (including possibilities of post-Congress trips).

NOTICE TO AUTHORS

ACTA PROTOZOOLOGICA is intended as a journal serving for the publication of original papers embodying the results of experimental or theoretical research in all fields of protozoology with the exception of faunistic notices of the local character and purely clinical reports. The papers should be concise and will not be accepted if they have been previously published elsewhere. After acceptance by the Editors papers will be printed in the possibly shortest time.

Papers are accepted in English, French, German and Russian. Every paper should begin with the name and postal address of the laboratory, name and the surname of the author and title in the language of the text. The titles in German and French should be translated into English according to the demands of Current Contents. The paper should be accompanied by synopsis in the language of the text not exceeding 100 words and by short summary in one of 4 languages accepted in the Journal. In the Russian text also the name and the postal address of the laboratory, legends of tables, plates and text illustrations must be translated, the translation of the summary may be somewhat more extensive, and the name of the author should be given additionally also in the Latin characters.

Manuscripts should be doublespaced typescript (30 lines on one side of a sheet) with a normal margin. No elements of the text should be fully typed in capitals nor in spaced set (only underlining with pencil is admittable). In decial fractions points not commas should be used. The generally accepted abbreviations and symbols are recomended. Nomenclature must agree with the International Code of Zoological Nomenclature, London 1961. The original and one carbon copy of the whole text material should be supplied.

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Galley proofs are sent to the authors. Authors receive 75 reprints without covers.

In preparation:

S. D. Amoji and M. J. Devdhar: *Echinospora phalangii* gen. n., sp. n. An Actinocaphaid Gregarine Found in the Intestine of Phalangid Host, *Opalnia* sp. — A. Czapik: *Frontonia pallida* sp. n. un nouveau cilié psammophile (*Hymenostomata, Peniculina*) — Г. А. Штейн: Новые данные о паразитических инфузориях (*Peritricha, Urceolariiae*) с рыб бассейна Тихого океана — А. Л. Юдин: Механизмы дестабилизации наследственных признаков у амеб. I. Подавление генетического взаимовлияния ядер в гетерокарионах — А. Л. Юдин: Механизмы дестабилизации наследственных признаков у амеб. II. Наследуемые изменения, индуцированные некоторыми антибиотиками — А. Л. Юдин: Механизмы дестабилизации наследственных признаков у амеб. III. Наследуемые изменения, обусловленные трансплантационной несовместимостью с амебами других штамов — E. Mikołajczyk and B. Diehn: Mechanosensory Responses and Mechanoreception in *Euglena gracilis* — S. A. Burnasheva and G. A. Solovjeva: Cytochemical Localization of Ca^2-ATPase Activity in Cilia and Basal Bodies of *Tetrahymena pyriformis* — Л. К. Лозина-Лозинский: Адаптивное поведение *Paramecium caudatum* при фотодинамическом действии красителей — Sisinthy Shivaji, D. M. Saxena and M. K. K. Pillai: — Effect of Metepa on the Growth of *Tetrahymena pyriformis* (Ciliata)

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