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Материалы по морфологии некоторых псаммофильных инфузорий Каспийского моря.

Materials on morphology of some psammophilic ciliates of the Caspian Sea

Фауна псаммофильных инфузорий (*Ciliata*) широко распространена в морях Мирового океана и отмечена также в пресных водоёмах. Однако пресноводный псаммон менее специфичен и в настоящее время слабее изучен, чем морской. Результаты многочисленных исследований показывают, что интерстициальная фауна достигает своего наивысшего развития в морском песке.

За последние годы подробно изучалась интерстициальная фауна инфузорий многих морей СССР (Баренцево, Белое, Японское и Черное), Западной Европы, Северной и Экваториальной Атлантики. В результате этих исследований выявлена богатейшая фауна интерстициальных инфузорий, а также описано довольно значительное число новых видов, что внесло ощутимый вклад в систематическую зоологию. В этом плане большой интерес представляет изучение морей с высокой степенью эндемизма фауны. Одним из таких морей является Каспийское.

Нами в псаммоне Каспийского моря был обнаружен 131 вид инфузорий. Среди них новыми оказались 14 видов, относящихся к семействам *Enchelyidae, Trachelocercidae, Loxodidae, Euplotidae* и *Aspidiscidae.*

Описания всех новых видов даны нами в предыдущих работах (Агамалиев 1966 a, 1966 b, 1967). В этой статье мы даём описания некоторых видов, характерных для интерстициальной фауны Каспия, морфология которых оставалась недостаточно изученной.

Инфузории были собраны на западном побережье Среднего и Южного Каспия. Изучение морфологии инфузорий производилось на препаратах, импрегнированных серебром по Шаттону и Львову (Chatton et Lwoff 1930), а также окрашеных по Фельгену или железным гематоксилином.

Работа выполнена в лаборатории цитологии одноклеточных организмов Института цитологии АН СССР.

Автор искренне благодарен И. Б. Райкову за ценные советы в работе.

Placus striatus Cohn, 1866 (Рис. 1, Табл. I 1)

Этот вид впервые был найден Коном (Cohn 1866); позже Каль (Kahl 1933) обнаружил его в песчаном грунте Кильской бухты (западное побережье Балтийского моря). Бок (Bock 1952) также обнаружил этот вид в этом же районе, а Дражеско (Dragesco 1963 a) — в песчаном грунте района биологической станции в Роскове. Нами этот вид был обнаружен в песке пляжа Бильги (северный берег Апшеронского полуострова) и в песках побережья Среднего Каспия. Описание этого вида даётся на основании прижизненных наблюдений и препаратов, импрегнированных серебром по методике Шаттона и Львова.

Форма тела вытянутая, овальная, с закругленными концами (Рис. 1 A, Табл. I 1). Ротовое отверстие расположено апикально и вооружено пучком трихитов, представляющих собой настоящий палочковый аппарат. Это образование занимает почти $^2/_3$ всей длины тела животного. Имеется одна крупная сократительная вакуоль, расположенная на заднем конце тела.

Ресничный покров обнаруживает сильную спирализацию и состоит из 18—20 ресничных рядов на каждой стороне тела. Следует отметить, что сильная спирализация ресничных рядов затрудняет точное определение их числа. Кинетосомы крупные, тесно сближены друг с другом. Ресничные ряды сопровождаются мощными мионемами (Рис. 1 A).

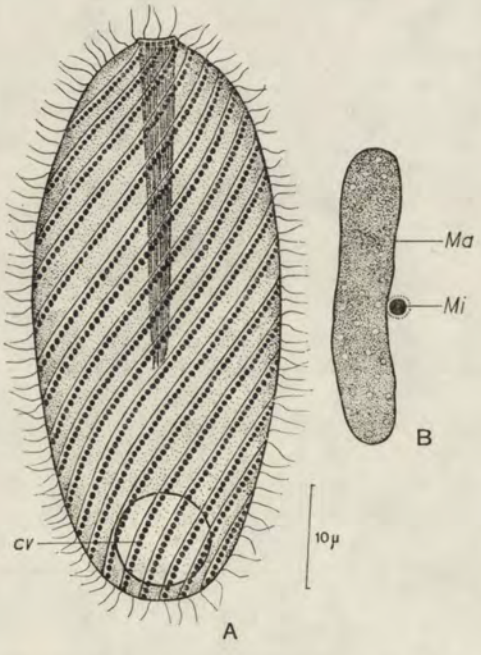

Рис. 1. *Placus striatus* Cohn, 1886. A — общий вид (серебрение); B — ядра (гемалаун), *Ma* — макронуклеус, *Mi* — микронуклеус, *cv* — сократительная вакуоль

Fig. 1. *Placus striatus* Cohn, 1886. A — general view (silver impregnation), B — nuclear apparatus (haemalaun), *Ma* — macronucleus, *Mi* — micronucleus, *cv* — contractile vacuole

Ядерный аппарат состоит из одного колбасовидного макронуклеуса длиной около 30 μ и одного сферического микронуклеуса (Рис. 1 В).

Длина тела фиксированных особей составляет 50—60 μ, живых — не более 100 μ. Питается в основном диатомовыми водорослями.

По всем признакам каспийская форма *P. striatus* идентична формам вышеуказанных авторов, за исключением морфологии макронуклеуса, который у наших форм более вытянут.

Биотоп: мелкий гомогенный песок Каспийского моря (микропоральный вид).

Prorodon binucleatus v. Buddenbrock, 1920 (Рис. 2)

Prorodon binucleatus отмечен Калем (K a h l 1930) как пресноводная форма. Дражеско (D r a g e s c o 1966), однако, нашел его в Средиземном море среди водорослей. В оригинальных описаниях указаных авторов нет сведений об его цилиатуре, что вызывает необходимость его переописания. Описание этого вида даётся на основании препаратов, серебренных по методике Шаттона и Львова и на основании прижизненных наблюдений. Вид найден в песке островов Бакинского архипелага в Южном Каспии.

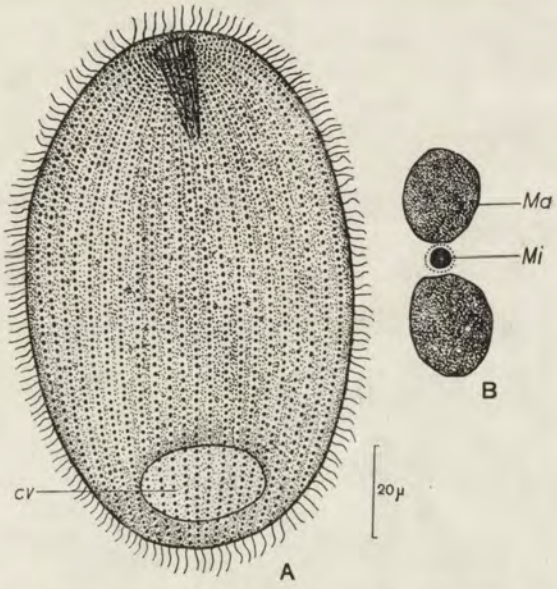

Рис. 2. *Prorodon binucleatus* v. Budd., 1920. А — общий вид (серебрение), В — ядра (гемалаун), *Ma* — макронуклеус, *Mi* — микронуклеус, *cv* — сократительная вакуоль

Fig. 2. *Prorodon binucleatus* v. Budd., 1920. A — general view (silver impregnation), B — nuclear apparatus (haemalaun), *Ma* — macronucleus, *Mi* — micronucleus, *cv* — contractile vacuole

Форма тела живых инфузорий вытянуто-цилиндрическая. В падающем свете особи коричневато-белые. Цитоплазма зернистая, непрозрачная. При фиксации инфузории получают симметрично-овальную форму (Рис. 2 А).

Ротовое отверстие расположено апикально, имеет воронкообразную форму и вооружено пучком трихитов. Задний конец тела закруглен и несет одну крупную сократительную вакуоль.

Ресничный покров густой, одевает все тело инфузории и состоит из большого числа (60—70) меридиональных рядов. Последние на всем протяжении тела инфузории идут параллельно друг другу и на переднем конце тела заканчиваются у ротового отверстия.

Ядерный аппарат состоит из двух почти шаровидных макронуклеусов и расположенного между ними одного микронуклеуса (Рис. 2 В).

Длина тела фиксированных особей составляет 100—110 μ, живых не более 150—200 μ. Питается в основном диатомовыми водорослями.

Биотоп: мелкий и средний песок Каспийского моря.

Lacrymaria coronata Clap. et Lachm., 1858 (Рис. 3)

Эта инфузория широко распространена на западном побережье Каспия. Краткое её описание приводится Калем (K a h l 1933). Бок (B o c k 1952) обнаружил этот вид в сапробных песках Кильской бухты. Позже Д р а ж е с к о обнаружил его в песчаном грунте района биологической станции в Роскове (D r a g e s c o 1960, 1966), в Средиземном море (1966) и в песках Африканского побережья экваториальной Атлантики (1965). Описание этого вида даётся нами на основании прижизненных наблюдений и серебрённых материалов.

Тело инфузории веретенообразное, слегка уплощенное дорзо-вентрально. Живые инфузории в падающем свете непрозрачны, цитоплазма забита раз-

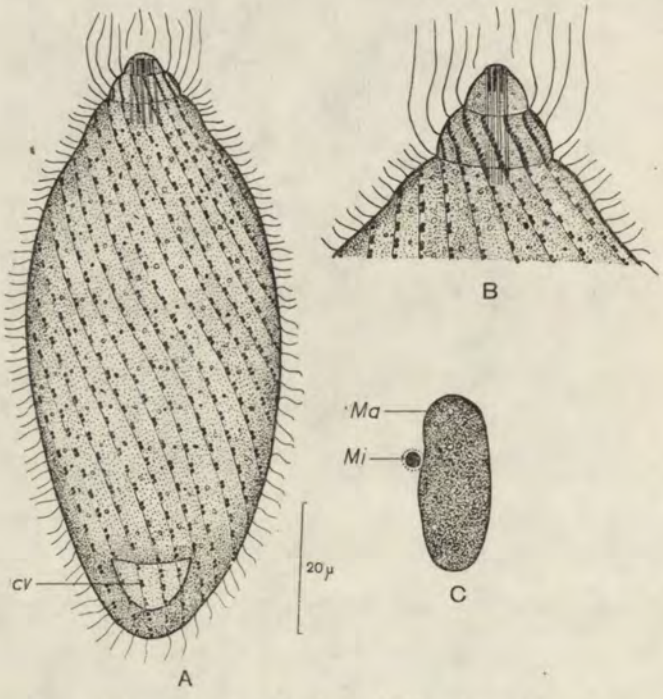

Рис. 3. *Lacrymaria coronata* Clap. et Lachm., 1858. A — общий вид, B — головка (A и B серебрение), C — ядра (гемалаун), *Ma* — макронуклеус, *Mi* — микронуклеус, *cv* — сократительная вакуоль

Fig. 3. *Lacrymaria coronata* Clap. et Lachm., 1858. A — general view, B — anterior end of body, the "head" (A and B silver impregnation), C — nuclear apparatus (haemalaun), *Ma* — macronucleus, *Mi* — micronucleus, *cv* — contractile vacuole

ными включениями. Спереди тело плавно переходит в короткую шейку, длиной около 15 μ и кончается хоботком. У фиксированых особей шейка выражена очень слабо (Рис. 3 A). Ротовые трихиты хорошо видны у фиксированных особей. Рот окружен венчиком длинных ресничек (Рис. 3 B). Задний конец тела слегка заострен и несет одну простую сократительную вакуоль.

Ресничный покров густой, равномерный, спирально покрывает все тело инфузории и состоит из 30—35 меридиональных рядов ресничек. На переднем конце тела ресничные ряды почти не образуют спирали. Кинетосомы, как показано на рисунках, расположены попарно.

Ядерный аппарат состоит из одного овального макронуклеуса, длиной около 15 μ, шириной 7 μ. Микронуклеус сферический и расположен близко от макронуклеуса (Рис. 3 C).

Длина тела фиксированных особей 90 μ, ширина 50 μ; прижизненно длина не более 180—200 μ. При серебрении нами обнаружены конъюгирующие особи, которые соединяются передними концами (Табл. I 2).

Биотоп: мелкий песок (микропоральный вид). В большом количестве встречается в районах Среднего и Южного Каспия. Почти по всем признакам каспийская форма идентична формам, описанным в литературе; отличие состоит только в сравнительно более сильной спирализации ресничных меридианов у нашей формы.

Tracheloraphis teissieri Dragesco, 1960 (Рис. 4)

Этот вид часто встречается на западном побережье Каспийского моря и характеризуется сравнительно малыми размерами тела. Вид впервые был найден Дражеско (Dragesco 1960) в песчаном грунте района биологической станции в Роскове. Позже, в 1963 году, этот автор вновь описал его с некоторыми дополнениями, касающимися морфологии ресничного и ядерного аппаратов.

По данным Дражеско, эта инфузория имеет 9—12 рядов ресничек, 18—26 макронуклеусов и 6—8 микронуклеусов. В 1965 году тот же автор в работе по исследованию Атлантического побережья Экваториальной Африки отмечает, что этот вид имеет 12—14 ресничных рядов, 22—23 макронуклеуса, 6—9 микронуклеусов.

Тело ланцетовидное, сплющено дорзо-вентрально, спереди переходит в очень длинную и тонкую шейку (Рис. 4 A). Живые инфузории в падающем свете коричневато-белые. Эктоплазма забита зернистыми включениями. Сократимость сильная. Передний конец тела не образует расширенной головки и забит черными светопреломляющими гранулами. Рот воронкообразный, терминальный, с продольной щелью, окружен гиалиновым утолщенным валиком и венчиком длинных ресничек (Рис. 4 A, B). Специализированных околоротовых трихоцист или трихитов не обнаружено. Задний конец тела заострен, но не образует вытянутого хвоста.

Ресничный покров состоит из 8—10 меридиональных рядов. По спинной стороне вдоль тела проходит узкая (по ширине равная всего 3—4 меридианам) голая полоска, характерная для рода *Tracheloraphis*. Между ресничными рядами, а также на голой полоске беспорядочно расположены протрихоцисты овальной или круглой формы (Рис. 4 C). У фиксированных особей протрихоцисты не сохраняются.

Ядерный аппарат состоит из 15—21 макронуклеуса и 4—8 микронуклеусов, расположенных в средней части тела в виде продольного ряда (Рис. 4 D). Макронуклеусы (Рис. 4 E) сферические, мелкие, диаметром 4—5 μ, содержат

нуклеолу, окруженную хроматиновыми гранулами. Микронуклеусы сферические, как обычно, ярко окрашиваются по Фельгену (Рис. 4 E).

Длина тела в вытянутом состоянии 200—400 μ, в фиксированном — 90—100 μ. Питается в основном диатомовыми водорослями.

Биотоп: очень мелкий и мелкий песок Каспийского моря.

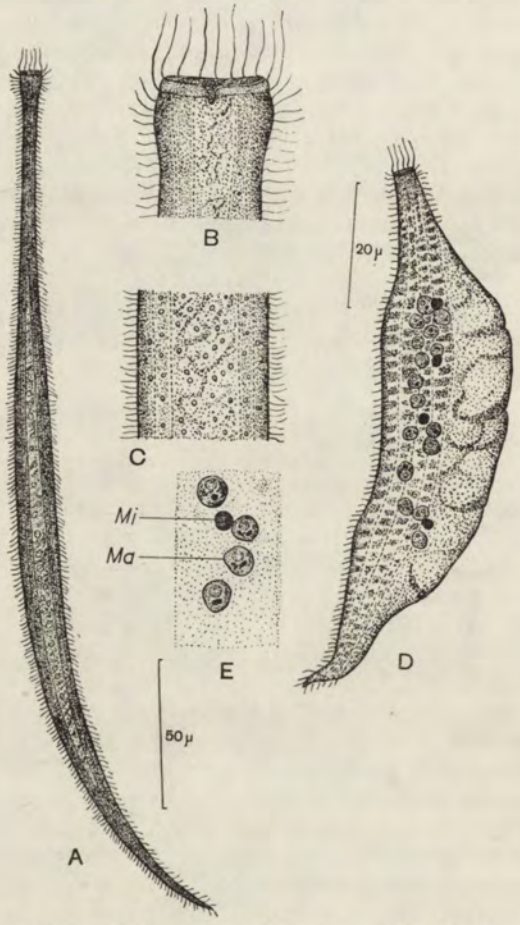

Рис. 4. *Tracheloraphis teissieri* Dragesco, 1960. A — общий вид (прижизненно), B — передний конец (прижизненно), C — трихоцисты, вид с поверхности тела, D — фиксированная особь, E — ядра (гемалаун), *Ma* — макронуклеус, *Mi* — микронуклеус

Fig. 4. *Tracheloraphis teissieri* Dragesco, 1960. A — general view of the living specimen, B — anterior end of the living specimen, C — trichocysts, the surface aspect, D — fixed specimen, whole preparation, E — nuclear apparatus (haemalaun), *Ma* — macronucleus, *Mi* — micronucleus

Paraspathidium fuscum (Kahl, 1928) (Рис. 5, Табл. I 3, 4)

Эта инфузория в настоящее время хорошо известна. Вначале Каль (Kahl 1928, 1930) отнес её к роду *Trachelocerca*. Форе-Фремье (Fauré-Fremiet 1951) и Дражеско (Dragesco 1960, 1963 a) находили её в песках Атлантического

и Средиземноморского побережья Франции (в Роскове, Конкарно, Баньюльсе). Кроме того, этот вид был также найден во всех остальных изученных географических районах. Нам этот вид встретился в массовом количестве на западном побережье Каспия. Описание даётся на основании препаратов, серебренных по методике Шаттона и Львова.

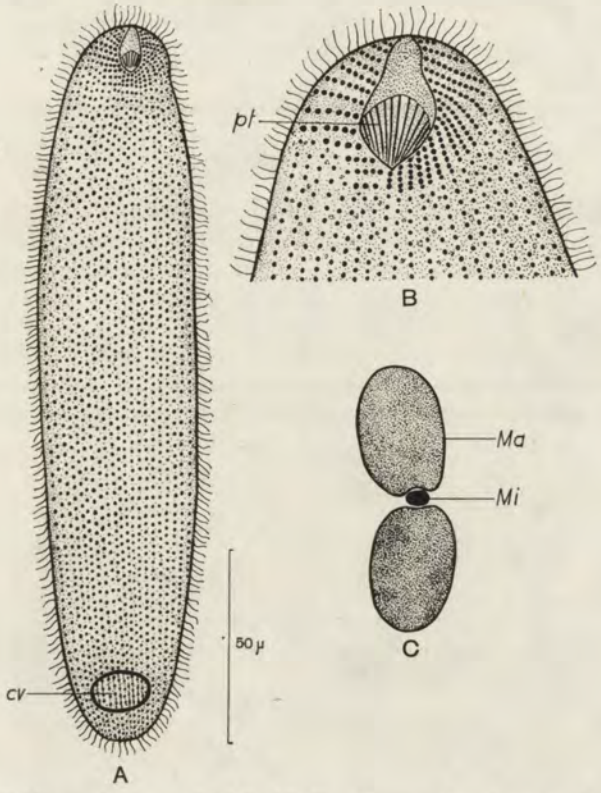

Рис. 5. *Paraspathidium fuscum* (Kahl, 1928). A — общий вид, B — передний конец (A и B серебрение), C — ядра (реакция Фельгена), *Ma* — макронуклеус, *Mi* — микронуклеус, *pt* — околоротовые трихиты, *cv* — сократительная вакуоль

Fig. 5. *Paraspathidium fuscum* (Kahl, 1928). A — general view, B — anterior end (A and B — silver impregnation), C — nuclear apparatus (Feulgen reaction), *Ma* — macronucleus, *Mi* — micronucleus, *pt* — paraoral trichites, *cv* — contractile vacuole

Форма тела веретенообразная или цилиндрическая, с закругленными концами (Рис. 5 A, Табл. I 3, 4). Рот довольно большой, расположен субтерминально на переднем конце тела (слегка смещен на брюшную сторону) и снабжен околоротовыми трихитами. Последние хорошо видны у фиксированных особей. Имеется одна терминальная сократительная вакуоль.

Ресничный покров густой, состоит из 40—45 меридиональных рядов. Ресничные ряды равномерно покрывают все тело инфузории и идут параллельно друг другу. На переднем конце тела ресничные ряды, как показано на рисунках, имеют очень характерное расположение (Рис. 5 B, Табл. I 3). Кинетосомы здесь крупные, а реснички длиннее, чем на остальной части тела.

Ядерный аппарат обычно состоит из 2 овальных макронуклеусов и расположенного между ними одного круглого микронуклеуса (Рис. 5 С). Однако нам встречались особи, ядерный аппарат которых состоял из четырех макронуклеусов, компактно расположенных в средней части тела. Кроме того, в редких случаях встречались особи, имеющие один или три макронуклеуса. Цитоплазма непрозрачная, забита разными включениями. Питается в основном диатомовыми и другими одноклеточными водорослями. Сократимость сильная.

Длина тела у фиксированных особей 150—200 μ, прижизненно — не более 400—500 μ.

Биотоп: мелкий и средний песок Каспийского моря (мезопоральный вид). Эта инфузория довольно часто встречается в массовом количестве в сапробном песке.

Frontonia marina Fabre-Domergue, 1891 (Рис. 6, Табл. II 5, 6)

Очень характерная форма, отмеченная во всех изученных географических районах. Нами она была обнаружена на западном побережье Среднего и Южного Каспия. В массовом количестве она встречается в песках островов Бакинского архипелага.

Форма тела овальная (Рис. 6 А, Табл. II 5, 6)). Живые инфузории в падающем свете коричневато-белые. Цитоплазма непрозрачная, забита диатомовыми водорослями и другими включениями. Задний конец тела снабжен сравнительно

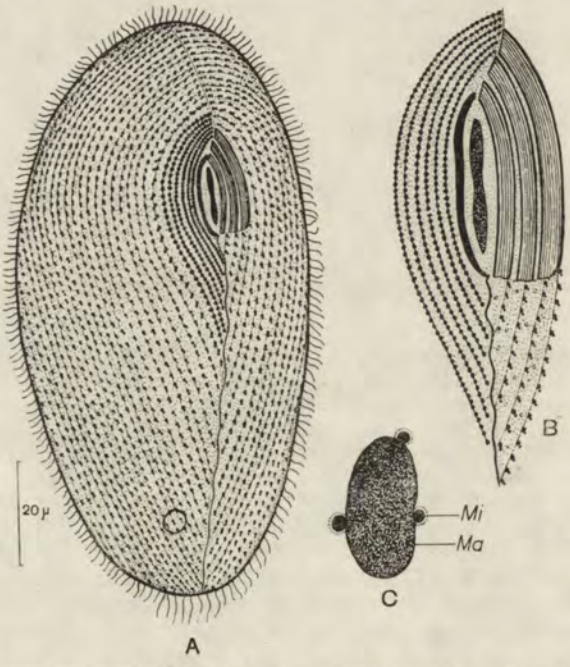

Рис. 6. *Frontonia marina* Fabre-Domergue, 1891. А — общий вид с брюшной стороны, В — ротовый аппарат (А и В серебрение), С — ядерный аппарат (гемалаун), *Ma* — макронуклеус, *Mi* — микронуклеус

Fig. 6. *Frontonia marina* Fabre-Domergue, 1891. A — general view of the ventral side, B — buccal apparatus (A and B — silver impregnation), C — nuclear apparatus (haemalaun), *Ma* — macronucleus, *Mi* — micronucleus

более длинными ресничками и несёт одну простую сократительную вакуоль. Брюшная сторона тела плоская, а спинная сторона слегка выпуклая (Табл. II 5, 6).

Рот расположен вблизи переднего конца тела; длина предротовой ямки (вестибулюма) 20 μ, а ширина 5—10 μ. Справа от цитостома расположена ундулирующая мембрана и шесть перизональных[1] рядов ресничек, сближенных между собою. В отличие от других меридиональных рядов ресничек, эти ряды при серебрении окрашиваются темнее, кинетосомы их крупнее и расположены очень тесно друг к другу (Рис. 6 A, B, Табл. II 5). Перизональные ряды ресничек упираются спереди в предротовой шов, сзади — в вентральный шов.

В левой части вестибулюма расположены три пеникулуса (сложные ресничные образования, состоящие из нескольких тесно сближенных рядов кинетосом). Имеется четыре посторальных ресничных ряда, которые в дальнейшем упираются в вентральный шов.

Ресничный покров состоит из 100—130 меридиональных рядов. Передние концы этих рядов упираются в предротовой шов. Часть из них кончается на вентральном шве, а часть рядов параллельно проходит до заднего конца тела (Рис. 6 A). На спинной стороне тела ресничный покров, как показано на Табл. II 6, имеет очень характерное расположение.

Ядерный аппарат состоит из одного макронуклеуса и 1—3 микронуклеусов (Рис. 6 C).

Длина тела у фиксированных особей составляет 100—130 μ, живых не более 150—200 μ

Биотоп: мелкий, средний и крупный песок Каспийского моря.

Frontonia macrostoma Dragesco, 1960 (Табл. II 7)

Этот вид впервые был найден Дражеско (D r a g e s c o 1960) в песке района биологической станции в Роскове. Нами он был обнаружен в районе Апшеронского полуострова и островов Бакинского архипелага.

Форма тела овальная (Табл. II 7), цитоплазма забита разными включениями. Рот расположен в середине тела. Справа от цитостома расположена ундулирующая мембрана и 5 рядов перизональных меридианов, сближенных между собою. В левой части цитостома имеется три пеникулуса. На заднем конце рта начинается 4—5 посторальных рядов ресничек, которые в дальнейшем упираются в вентральный шов.

Ресничный покров состоит из 70—90 меридиональных рядов.

Ядерный аппарат состоит из одного макронуклеуса и одного микронуклеуса.

Длина тела 100—150 μ. Имеется одна крупная терминальная сократительная вакуоль.

Биотоп: средний и крупный песок Каспийского моря.

Pleuronema coronatum Kent, 1881 (Рис. 7, Табл. III 8, 9)

Pleuronema coronatum часто встречается в песках западного Каспия. Описания её даются Калем (K a h l 1930—1935), Форе-Фремье (F a u r é - F r e m i e t 1950), Дражеско (D r a g e s c e 1960, 1963 a, 1965), Боррором (B o r r o r 1963) и другими авторами. Мы описываем этот вид на основании серебренных материалов.

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[1] Термин „перизональная цилиатура", предложенный А. В. Янковским (J a n k o w s k i 1964 a, 1964 b), обозначает сближенные соматические ресничные ряды, прилегающие к перистому. Функция перизональной цилиатуры сводится к усилению тока воды в районе перистома, что способствует более активному питанию инфузорий.

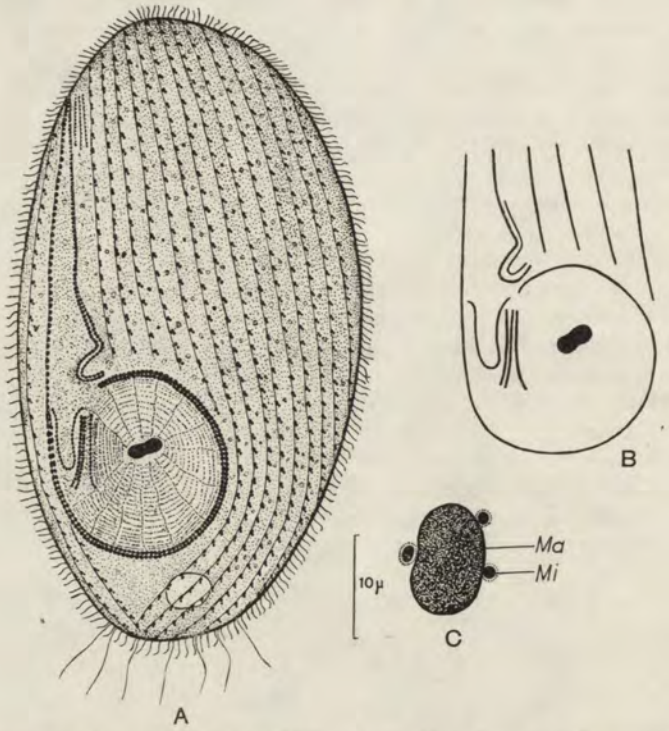

Рис. 7. *Pleuronema coronatum* Kent, 1881. A — общий вид с брюшной стороны, B — буккальный аппарат (A и B серебрение), C — ядерный аппарат (гемалаун), *Ma* — макронуклеус, *Mi* — микронуклеус

Fig. 7. *Pleuronema coronatum* Kent, 1881. A — general view of the ventral side, B — buccal apparatus (A and B — silver impregnation), C — nuclear apparatus (haemalaun), *Ma* — macronucleus, *Mi* — micronucleus

Форма тела яйцевидная или овальная (Рис. 7 A, Табл. III 8). Цитоплазма бесцветная, забита диатомовыми водорослями и другими включениями. Задний конец тела снабжен удлиненными ресничками или щетинками и несет терминальную сократительную вакуоль. Буккальная полость спереди узкая, а позади округлая, диаметром около 20 μ. Ундулирующая мембрана занимает больше половины тела. Длина её составляет около 50—60 μ. Спереди на краю буккальной полости оканчиваются 4—5 ресничных меридианов, идущих параллельно от переднего конца тела. Буккальный ресничный аппарат характерен для вида *P. coronatum* (Рис. 7 B, Табл. III 8).

Ресничный покров, включая 4—5 предротовых меридианов, состоит из 30—35 ресничных рядов.

Ядерный аппарат у нашей формы состоит из одного сферического или овального макронуклеуса диаметром около 10 μ и 3—5 микронуклеусов, расположенных вокруг макронуклеуса (Рис. 7 C, Табл. III 9).

Длина тела у фиксированных особей составляет 60—70 μ, а ширина около 40 μ.

Биотоп: мелкий и средний песок Каспийского моря. Встречается также в скоплениях диатомовых водорослей.

Pleuronema setigerum Calkins, 1903

Описания этого вида даются Калем (Kahl 1930—1935) и Боррором (Borror 1963). Этот вид встретился нам в песках Апшеронского побережья Каспия. По своей форме тела он похож на *P. coronatum*, отличается от него слегка расширенным задним концом и меньшим числом ресничных меридианов. Задний конец тела несёт одну простую сократительную вакуоль. Буккальный ресничный аппарат имеет в длину 40—50 μ. Спереди к ротовой полости подходят 3—4 ресничных ряда.

Ресничный покров состоит из 26—28 меридиональных рядов.

Ядерный аппарат состоит из одного макронуклеуса и одного микронуклеуса. Длина тела 50—60 μ.

Биотоп: мелкий песок Каспийского моря.

Peritromus faurei Kahl, 1932

Вид впервые был найден Калем (Kahl 1932). Позже он отмечен во всех изученных географических районах.

Форма тела округлая, сплющенная в дорзо-вентральном направлении. Мало подвижная инфузория. Адоральная зона мембранелл занимает передний конец инфузории, спускается по левому краю и доходит почти до середины тела. Она состоит из 40—45 мембранелл. Цитоплазма гранулярная, забита диатомовыми водорослями и другими включениями.

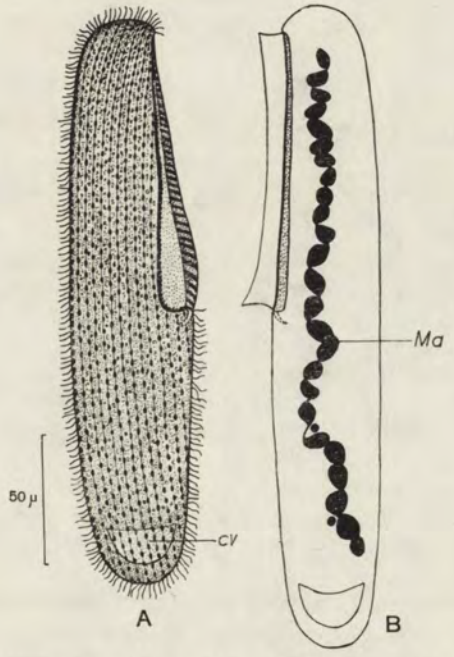

Рис. 8. *Blepharisma clarissimum* Anigstein, 1912. A — общий вид с правой стороны тела (серебрение), B — ядра (реакция Фельгена), *Ma* — макронуклеус, *cv* — сократительная вакуоль

Fig. 8. *Blepharisma clarissimum* Anigstein, 1912. A — general view of the right side of body (silver impregnation), B — nuclear apparatus (Feulgen reaction), *Ma* — macronucleus, *cv* — contractile vacuole

Ресничный покров одевает только брюшную сторону инфузории. Он состоит из 17—20 меридиональных рядов. Спинная сторона тела плоская и снабжена многочисленными короткими щетинками, которые хорошо видны даже на окрашенных препаратах.

Ядерный аппарат состоит из 2 макронуклеусов и 2—5 микронуклеусов, расположенных вокруг макронуклеусов. Длина тела 70—100 μ.

Биотоп: мелкий гомогенный песок Каспийского моря.

Blepharisma clarissimum Anigstein, 1912 (Рис. 8, Табл. III 10, 11)

Описание этого вида дано Калем (Kahl 1930—1935), Форе-Фремье (Fauré-Fremiet 1950) и Райковым 1960. Часто встречается в песках западного Каспия. Описание даётся на основании препаратов, серебренных по методике Шаттона и Львова. Тело вытянуто в длину, сильно сплющено с боков (Рис. 8 А, Табл. III 10). Задний конец несет терминальную сократительную вакуоль. Цитоплазма бесцветная, прозрачная. Адоральная зона мембранелл тянется по брюшному краю тела и занимает почти половину тела, а у некоторых особей даже больше половины тела. Ундулирующая мембрана занимает правый край перистомальной полоски.

Ресничный покров равномерно одевает все тело инфузории и состоит из 24—30 меридиональных рядов ресничек (Рис. 8 А).

Макронуклеус имеет вид продольной цепочки узелков (Рис. 8 В, Табл. III 11). Количество узелков варьирует от 13 до 30.

Длина тела у фиксированных особей составляет 200—300 μ; у живых — не более 500—600 μ

Биотоп: мелкий и средний песок Каспийского моря (эврипоральный вид).

Euplotes gracilis Kahl, 1932 (Рис. 9, Табл. IV, 12)

Вид впервые был найден Калем (Kahl 1932) на острове Гельголанд. Дражеско (Dragesco 1963 b) обнаружил его в песках биологической станции в Роскове. Позже Тюффро (Tuffrau 1960) переописал этот вид на основании материалов, серебренных по методике Шаттона и Львова. Нами он обнаружен в песках Апшеронского побережья Каспийского моря. Описание даётся на основании серебренных материалов.

Форма тела овальная с симметричными краями (Рис. 9 А). Цитоплазма непрозрачная, бесцветная. Перистом занимает больше половины тела, длина его составляет около 30 μ. Адоральная зона состоит из 30—34 мембранелл. Брюшная сторона тела всегда имеет 9 фронто-вентральных, 5 трансверсальных и 3—4 каудальных цирр. Две задние фронто-вентральные цирры расположены близ правого края тела на одной продольной линии, как у *E. raikovi* и *E. strelkovi* (Агамалиев 1966 b, 1967). Вентральный аргиром такой же, как на оригинальном рисунке Тюффро (Tuffrau 1960). Имеется одна сократительная вакуоль.

В результате серебрения обнаруживаются 7 латерально-дорзальных рядов щетинок (Рис. 9А, В). Аргиром межщетинковых рядов очень своеобразен и покрывает всю дорзальную сторону инфузории в виде мелкой сетки (Рис. 9 В, Табл. IV 12).

Ядерный аппарат, как обычно, состоит из одного макронуклеуса и одного микронуклеуса (Рис. 9 С). Длина тела 50—55 μ.

Биотоп: мелкий гомогенный песок Каспийского моря.

Каспийская форма *E. gracilis* отличается от описанных в литературе форм

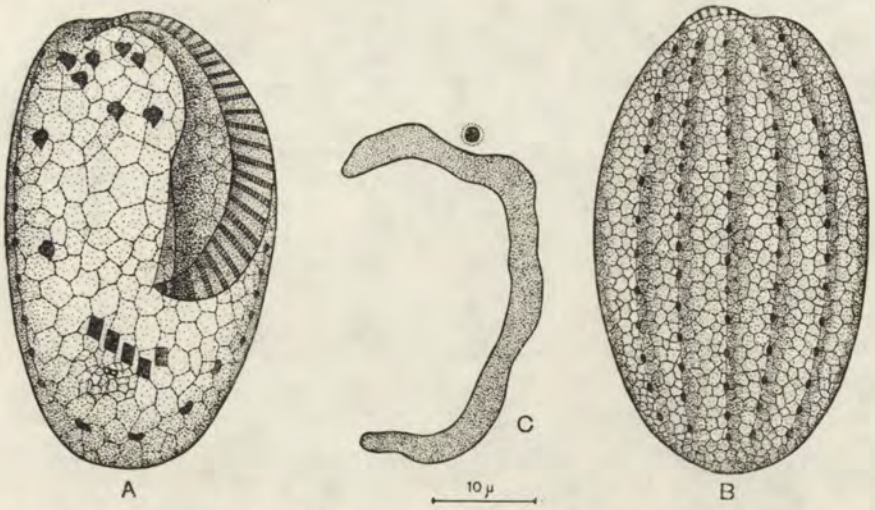

Рис. 9. *Euplotes gracilis* Kahl, 1932. A — общий вид с брюшной стороны, B — вид со спинной стороны (A и B серебрение), C — ядра (реакция Фельгена)
Fig. 9. *Euplotes gracilis*, Kahl, 1932. A — general view of the ventral side, B — view of the dorsal side (A and B — silver impregnation), C — nuclear apparatus (Feulgen reaction)

этого же вида несколько иным расположением фронто-вентральных цирр передней группы и вариабельностью числа каудальных цирр (3—4). По всем остальным признакам наша форма идентична формам, описанным в литературе.

Euplotes balteatus Dujardin, 1842 (?) (Рис. 10, 11, Табл. IV 13—15)

Описания *Euplotes balteatus* приводятся Калем (Kahl, 1932), Боррором (Borror 1963), Дражеско (Dragesco 1963 b), Тюффро (Tuffrau 1964). При работе на Каспийском море нами были обнаружены две формы этого вида инфузорий. Сравнение этих форм между собой и с формами, описанными в литературе, показало, что они по некоторым признакам (число каудальных цирр, дорзальных рядов щетинок, число щетинок на одном дорзальном ряду и число мембранелл адоральной зоны) заметно отличаются друг от друга (Таблица I). Поэтому мы даём описания обеих каспийских форм на основании материалов, серебренных по методике Шаттона и Львова.

Обе разновидности по своей форме тела и по вентральной цилиатуре, на первый взгляд, напоминают *Euplotes minuta* Yocom, 1930, переописанный Боррором (Borror 1962), но отличаются от последнего меньшим числом латерально-дорзальных форм щетинок и иным строением дорзального межщетинкового аргирома.

У первой формы тело овальное, цитоплазма бесцветная (Рис. 10 A). Перистом очень большой, занимает $^3/_4$ длины тела. Вентральный аргиром типа *E. eurystomus*. Имеется одна сократительная вакуоль. Как указано в Таблице 1, обнаружено 8—9 латерально-дорзальных рядов щетинок (Рис. 10 A, B, Табл. IV 13,14). Аргиром межщетинковых рядов типа *E. raikovi* (Агамалиев 1966 b).

Ядерный аппарат по своей форме ближе всего к ядерному аппарату *E. cri-*

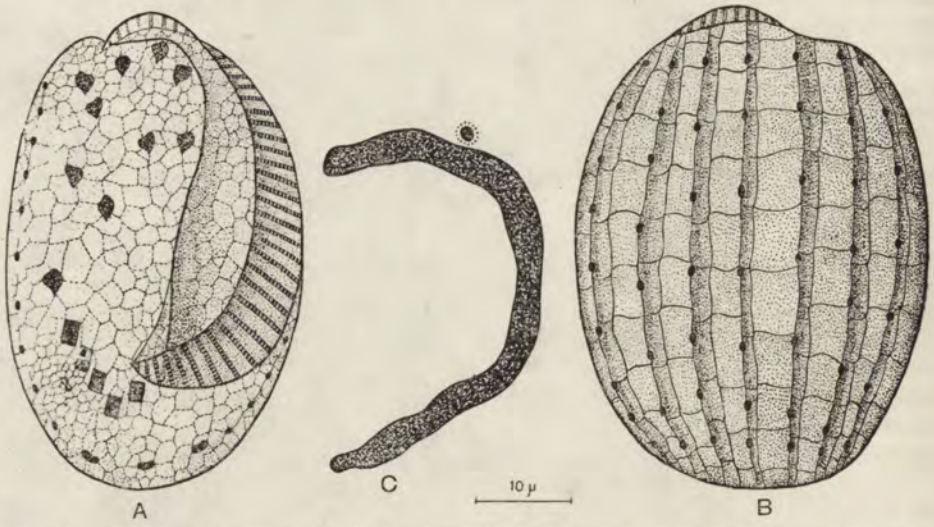

Рис. 10. *Euplotes balteatus* Dujardin, 1842. (1-ая форма). A — общий вид с брюшной стороны, B — вид со спинной стороны (A и B серебрение), C — ядра (гемалаун)

Fig. 10. *Euplotes balteatus* Dujardin, 1842, (first form), A — general view of the ventral side, B — view of the dorsal side (A and B — silver impregnation), C — nuclear apparatus (haemalaun)

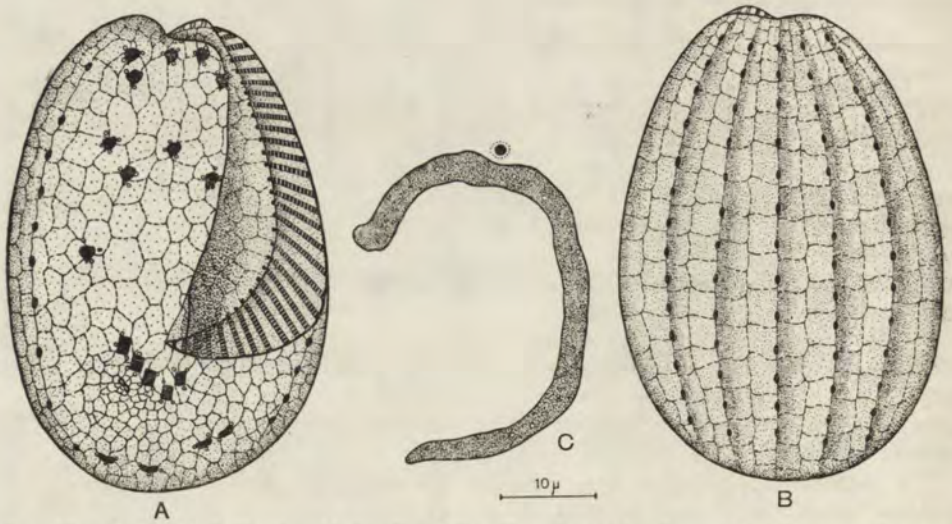

Рис. 11. *Euplotes balteatus* Dujardin, 1842. (2-я форма). A — общий вид с брюшной стороны, B — вид со спинной стороны (A и B серебрение), C — ядра (гемалаун)

Fig. 11. *Euplotes balteatus* Dujardin, 1842, (second form), A — general view of the ventral side, B — view of the dorsal side (A and B — silver impregnation), C — nuclear apparatus (haemalaun)

status; он состоит из одного макронуклеуса и расположенного вблизи от него одного микронуклеуса (Рис. 10 C).

Вторая форма *E. balteatus* отличается от первой формы тупо закругленным задним и слегка суженным передним концами (Рис. 11 A, B, Табл. IV 15). Цитоплазма прозрачная. Перистом занимает больше половины тела; длина его составляет 35 μ. Адоральная зона мембранелл занимает $^2/_3$ длины тела. Данные о вентральной цилиатуре и других морфологических признаках приведены в Таблице 1. Вентральный аргиром такого же типа, как у первой фор-

Таблица 1

Table 1

Морфологические признаки различных форм *Euplotes balteatus* Dujardin, 1842

Morphological features of different forms of *Euplotes balteatus* Dujardin, 1842

	Типичная форма Typical form	1-ая каспийская форма First caspian form	2-ая каспийская форма Second caspian form
Число фронто-вентральных цирр Number of fronto-ventral cirri	10	10	10
Число трансверсальных цирр Number of transversal cirri	5	5	5
Число каудальных цирр Number of caudal cirri	4	3—4	4
Число мембранелл адоральной зоны Number of maembranelles of adoral zone	30—35	35—40	28—33
Число латерально-дозальных рядов щетинок Number of latero-dorsal rows of bristles	8—10	8—9	7
Число щетинок на одном дорзальном ряду Number of bristles in one dorsal row	8—11	7—8	13—14
Длина тела в μ Length of body in μ	30—150	50—60	50

мы. Имеется одна сократительная вакуоль. Характерной чертой этой формы является число латерально-дорзальных рядов щетинок (всего 7) и число щетинок в одном дорзальном ряду. Ячейки межщетинкового аргирома такие же, как у формы, переописанной Тюффро (T u f f r a u 1964).

Ядерный аппарат, характерный для рода *Euplotes*, состоит из одного макронуклеуса и одного микронуклеуса (Рис. 11 C).

Биотоп: мелкий гомогенный песок Каспийского моря. Обе формы были обнаружены на южном берегу Апшеронского полуострова.

Как видно из Таблицы 1, каспийские формы *E. balteatus* почти по всем

признакам идентичны типичной форме, описанной в литературе (Borror 1963, Dragesco 1963 b, Tuffrau 1964). Отличие касается, как отмечено выше, каудальных цирр, дорзальных рядов щетинок и числа щетинок в одном дорзальном ряду. Так, в первой форме часто встречаются особи с 3-мя каудальными циррами. У других форм такое число каудальных цирр не встречается. Что касается второй формы, то она по своей форме тела, вентральной цилиатуре и по строению дорзального межщетинкового аргирома стоит ближе всего к типичным *E. balteatus*. Однако она отличается от типичной формы нехваткой одного из латерально-дорзальных рядов щетинок и большим числом щетинок в одном дорзальном ряду, а также сравнительно меньшим числом мембранелл адоральной зоны. Вполне допустима вариабельность указанных признаков, поэтому пока мы не можем выделить эти формы в самостоятельные виды. Для этого необходимо изучение изменчивости указанных признаков в клональных культурах.

Euplotes harpa Stein, 1859 (Рис. 12, Табл. V 16, 17).

Описания этого вида приводятся Калем (Kahl 1930—1935) и Тюффро (Tuffrau 1960). Нами он обнаружен в крупных и средних песках островов Бакинского архипелага (в Южном Каспии) и побережья Среднего Каспия.

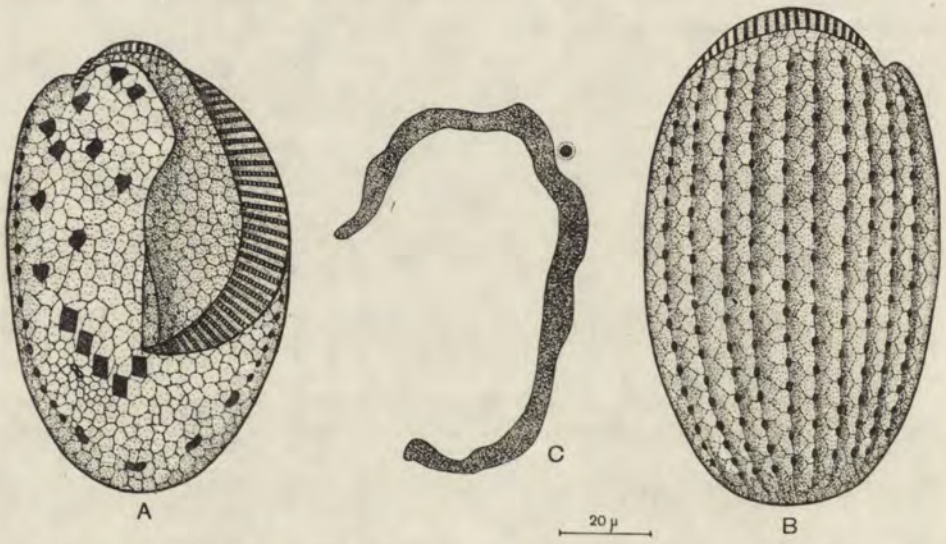

Рис. 12. *Euplotes harpa* Stein, 1859. A — общий вид с брюшной стороны, B — вид со спинной стороны (A и B серебрение), C — ядра (гемалаун)
Fig. 12. *Euplotes harpa* Stein, 1859. A — general view of the ventral side, B — view of the dorsal side (A and B — silver impregnation), C — nuclear apparatus (haemalaun)

Форма тела овальная (Рис. 12 A, Табл. V 16). Живые инфузории в падающем свете по краям тела прозрачны, а середина тела забита коричневыми включениями. Перистом большой, занимает $^2/_3$ длины тела. Длина его составляет 60—70 μ. Выступ на правой стороне перистомальной полости — характерный признак этого вида. Адоральная зона состоит из 52—60 мембранелл. Вентральная сторона тела имеет 10 фронто-вентральных, 5 трансвер-

сальных, 4—6 каудальных цирр. Иногда встречаются особи, которые имеют 3 каудальных цирры. Вентральный аргиром такой же, как у *E. eurystomus*. Имеется одна сократительная вакуоль. В результате серебрения обнаруживается 11—12 латерально-дорзальных рядов щетинок (Рис. 12 A, B Табл. V 16,17). Аргиром межщетинковых рядов типа *E. eurystomus*.

Ядерный аппарат состоит из одного С-образного макронуклеуса и одного микронуклеуса (Рис. 12 C).

Длина тела 90—100 μ. Встречаются особи длиной 130—150 μ.

Биотоп: крупный и средний олигосапробный песок Каспийского моря.

Каспийская форма *E. harpa* почти по всем признакам идентична формам, описанным в литературе. Однако следует отметить вариабельность числа каудальных цирр (3—6) и латерально-дорзальных рядов щетинок (11—12). По литературным данным, число каудальных цирр равно 4, а число латерально-дорзальных рядов щетинок — 13 .

Diophrys scutum Dujardin, 1842 (Рис. 13, Табл. V 18—19).

Типично морская форма. Отмечена во всех изученных географических районах. Описания приводятся в работах Каля (K a h l 1930—1935), Дражеско

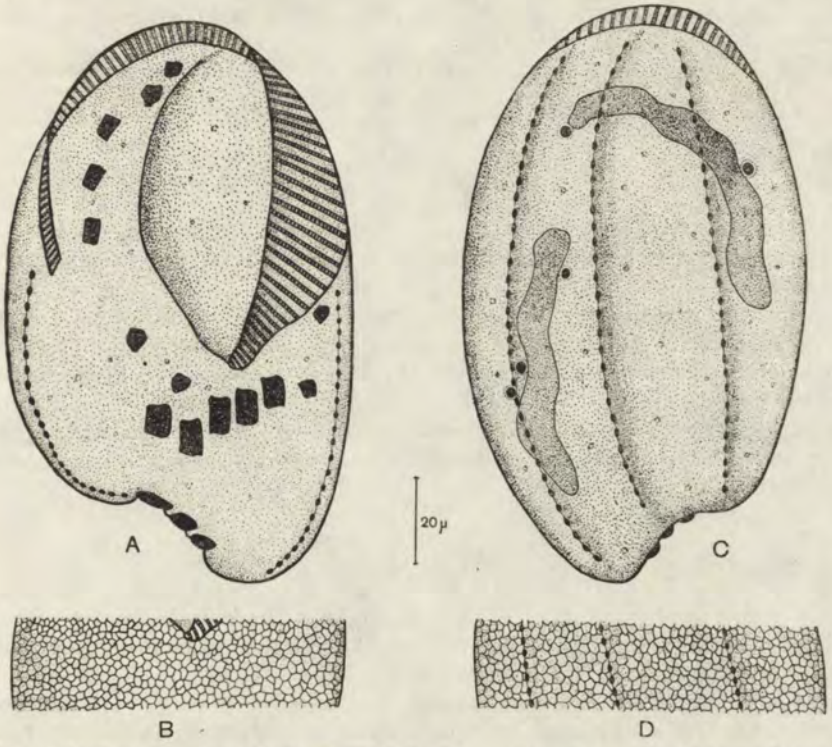

Рис. 13. *Diophrys scutum* Dujardin, 1842. A — общий вид с брюшной стороны, B — строение брюшного аргирома, C — общий вид со спинной стороны, D — спинной аргиром (A — D — серебрение)

Fig. 13. *Diophrys scutum* Dujardin, 1842. A — general view of the ventral side, B — ventral argyrome, C — general view of the dorsal side, D — dorsal argyrome (A — D — silver impregnation)

(Dragesco 1963 b) и Боррора (Borror 1963, 1965). Нам встретилась в большом количестве на островах Бакинского архипелага и на других участках западного побережья Каспийского моря.

Форма тела овальная, с закругленным передним концом (Рис. 13 A, Табл. V 18). Цитоплазма забита в основном диатомовыми водорослями, которые хорошо видны у фиксированных особей. Перистом занимает больше половины тела, длина его составляет 80 μ. Адоральная зона мембранелл начинается на правом крае тела, проходит по переднему краю перистомальной полости, спускается по левому краю тела до верхней границы трансверсальных цирр. Она состоит из 75—80 мембранелл. Вентральная поверхность тела имеет 7 фронто-вентральных, 5 трансверсальных, 2 левых и 3 правых маргинальных цирр (Рис. 13 A, Табл. V 18). Вентральный аргиром имеет вид сетки с мелкими ячейками (Рис. 13 B). В результате серебрения обнаруживаются 5—6 латерально-дорзальных рядов щетинок (Рис. 13 A—C, Табл. V 18, 19). Межщетинковый аргиром того же типа, что и вентральный аргиром данного вида (Рис. 13 D).

При фиксации нами обнаружены особи, готовящиеся к делению, у которых адоральная зона мембранелл еще не подвергалась изменениям.

Ядерный аппарат состоит из двух макронуклеусов и 5—6 микронуклеусов, расположенных рядом с макронуклеусами (Рис. 13 C).

Длина тела на фиксированном материале составляет 120—130 μ, прижизненно 150—170 μ.

Биотоп: средний и крупный олигосапробный песок; встречается также в мелком и очень мелком песке Каспийского моря.

Резюме

Изучены некоторые псаммофильные инфузории Каспийского моря с помощью импрегнации серебром по Шаттону и Львову, нуклеальной реакции Фельгена и окраски железным гематоксилином.

В статье даётся описание некоторых видов (*Placus striatus, Prorodon binucleatus, Lacrymaria coronata, Tracheloraphis teissieri, Paraspathidium fuscum, Frontonia marina, Frontonia macrostoma, Pleuronema coronatum, Pleuronema setigerum, Peritromus faurei, Blepharisma clarissimum, Euplotes gracilis, Euplotes balteatus, Euplotes harpa, Diophrys scutum*), наиболее характерных для интерстициальной фауны Каспия. Отмечена вариабельность некоторых морфологических празнаков у этих видов.

SUMMARY

Some psammophilic ciliates of the Caspian Sea were investigated using the silver impregnation method after Chatton and Lwoff, the Feulgen nuclear reaction and the iron haematoxylin staining method.

The description of the following species are included: *Placus striatus, Prorodon binucleatus, Lacrymaria coronata, Tracheloraphis teissieri, Paraspathidium fuscum, Frontonia marina, Frontonia macrostoma, Pleuronema coronatum, Pleuronema setigerum, Peritromus faurei, Blepharisma clarissimum, Euplotes gracilis, Euplotes balteatus, Euplotes harpa, Diophrys scutum.*

These are the most characteristic species for the interstitial fauna of the Caspian Sea. The variability of some morphological charaters of these species is reported.

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ПОДПИСЫ К ТАБЛИЦАМ I — V

1: *Placus striatus*, общий вид
2: *Lacrymaria coronata*, конъюгирующие особи
3—4: *Paraspathidium fuscum*, 3 — общий вид с брюшной стороны, 4 — общий вид со спинной стороны
5—6: *Frontonia marina*, 5 — общий вид с брюшной стороны, 6 — общий вид со спинной стороны
7: *Frontonia macrostoma*, общий вид с брюшной стороны
8—9: *Pleuronema coronatum*, 8 — общий вид с брюшной стороны, 9 — ядерный аппарат
10—11: *Blepharisma clarissimum*, 10 — общий вид, 11 — ядра
12: *Euplotes gracilis*, строение спинного аргирома
13—15: *Euplotes balteatus*, 13 — первая форма, общий вид с брюшной стороны, 14 — общий вид со спинной стороны, 15 — вторая форма, общий вид с брюшной стороны
16—17: *Euplotes harpa*, 16 — общий вид с брюшной стороны, 17— общий вид со спинной стороны
18—19: *Diophrys scutum*, 18 — общий вид с брюшной стороны, 19 — общий вид со спинной стороны
1 — 8, 10, 12 — 19: серебрение, 9 — гемалаун, 11 — реакция Фельгена

EXPLANATION OF PLATES I—V

1: *Placus striatus*, general wiev
2: *Lacrymaria coronata*, conjugation
3—4: *Paraspathidium fuscum*, 3 — general view of the ventral side, 4 — general view of the dorsal side
5—6: *Frontonia marina*: 5 — general view of the ventral side, 6 — general view of the dorsal side
7: *Frontonia macrostoma*, general view of the ventral side
8—9: *Pleuronema coronatum*, 8 — general view of the ventral side, 9 — nuclear apparatus
10—11: *Blepharisma clarissimum*, 10 — general view, 11 — nuclear apparatus
12: *Euplotes gracilis*, pattern of the dorsal argyrome
13—15: *Euplotes balteatus*, 13 — first form, general view of the ventral side, 14 — general view of the dorsal side, 15 — second form, general view of ventral side
16—17: *Euplotes harpa*, 16 — general view of the ventral side, 17 — general view of the dorsal side
18—19: *Diophrys scutum*, 18 — general view of the ventral side, 19 — general view of the dorsal side
1—8, 10, 12—19: silver impregnation, 9 — haemalaun, 11 — Feulgen reaction


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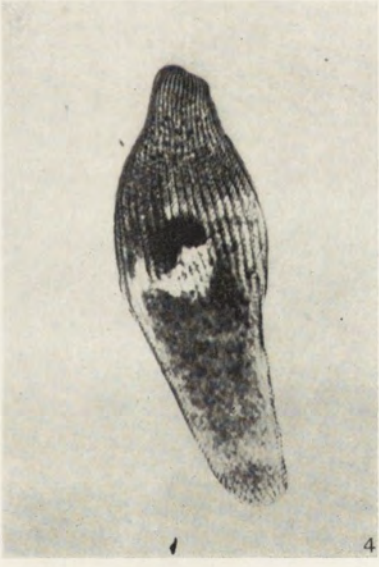
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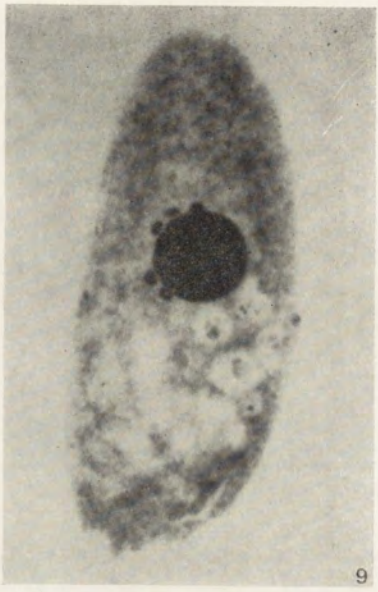
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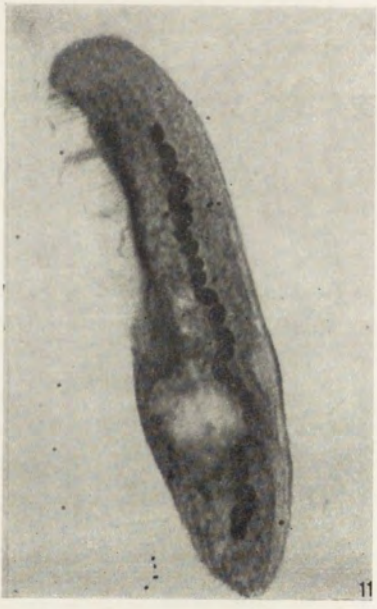
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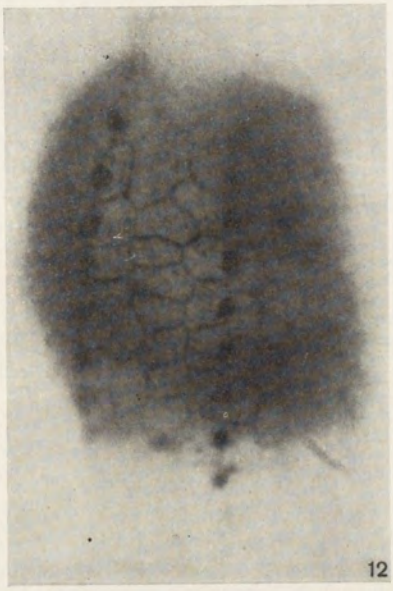
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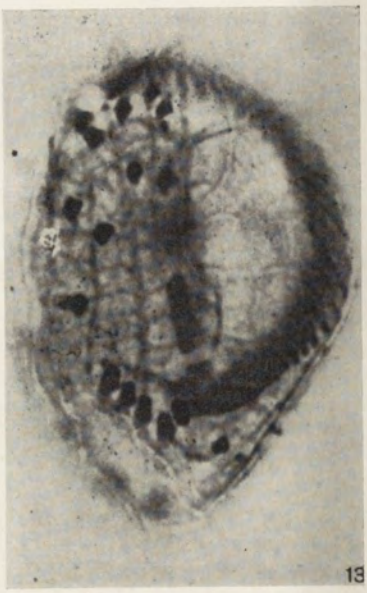
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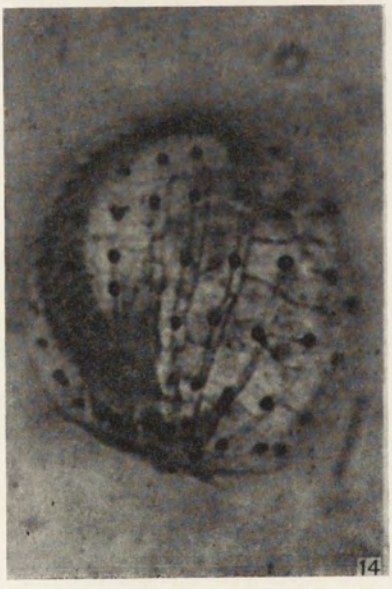
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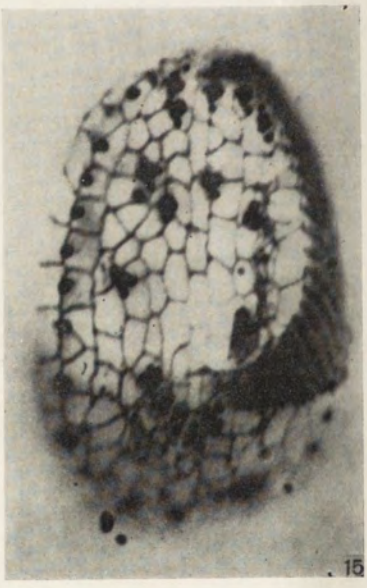
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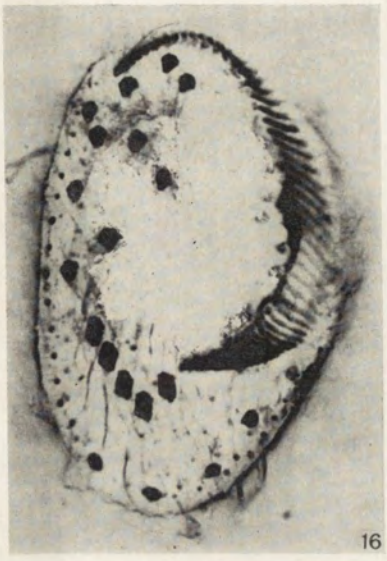
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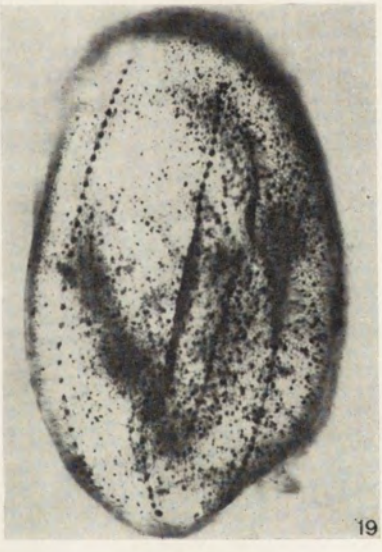
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Apiosoma from fresh-water fishes in the European part of the Soviet Union (systematic review)

Апиозомы пресноводных рыб Европейской части СССР (систематический обзор)

The family *Scyphidiidae* Kahl, 1935 includes the sessiline peritrichs (*Peritricha Sessilia*) occurring on the body surface of fishes. Until quite recently they were regarded as commensals, so they were only seldom noted during the examination of fishes for parasites. But during the last years it was found that they were genuine ectoparasites occurring in mass on the fry. There were found epizooties caused by these ciliates leading to the death of young carps in pond cultures (Fijan 1962, Rasmashkin i Skriptshenko 1965).

Detailed investigations of this group of ciliates are difficult because there is a lack of well marked specific characters in the whole family. Moreover, great variability of the forms included makes their descriptions inaccurate and determination of the species difficult.

Such a situation inclined the author to undertake investigations on sciphidians occurring on fresh-water fishes in the European part of USSR. The main aims of the present work were as follows: to establish the most conspicuous characters for taxonomy, to complete the descriptions, and to prepare the methodical ground for determination of the species.

The family *Scyphidiidae*, according to the system of Kahl, includes two genera: *Scyphidia* Dujardin, 1841 and *Glossatella* Bütschli, 1889. But, as it was stated by Lom 1966, the generic name *Glossatella* had to be rejected in favour of *Apiosoma* because the first representative of the genus had been described by Blanchard in the year 1885 under the name *Apiosoma piscicola* from the body surface of carps. This name will be used further in the present paper.

One of two genera, which compose the family *Scyphidiidae*, the genus *Scyphidia* occurs rarely and we shall not deal with it in the present paper. Contrary to it the genus *Apiosoma* is widely distributed.

Materials and methods

The researches were carried out on the representatives of the genus *Apiosoma* found in fresh-water reservoir of the European part of the Soviet

Union and in the valley of the river Vachš [1] in the Median Asia (one finding).

The smears fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin and the dry smears silver impregnated after Klein as well as living specimens were used for examination. The mass populations of *Apiosoma* were examined.

In order to investigate the character of variability and its ranges there were made the measurements of the length and width of the body and of the nuclei in 30—50 specimens from each population. The measurements were made on smears fixed in Schaudinn's fluid. They were compared with the measurements made on living ciliates from the same populations in order to reveal the presence and degree of deformations caused by fixation. The obtained data were analized statistically. The measurements in tables are given in microns.

Out of ten species examined four appeared to be new and the other four, described by T i m o f e e w and K a s h k o w s k i j, were lacking in complete descriptions. The redescriptions of these latter are also given.

The scheme of the body structure of *Apiosoma*, with the designations of all parts that will be used in the following descriptions, is given in Fig. 1.

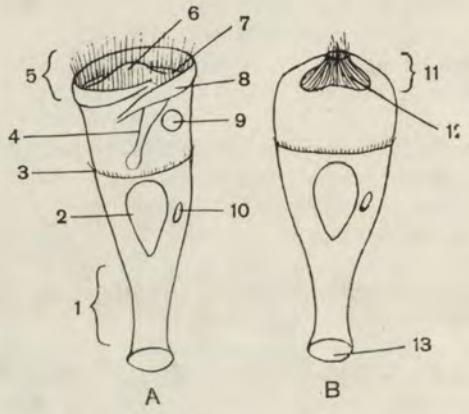

Fig. 1. Scheme of the structure of *Apiosoma*, A. specimen with spread peristome: 1 — stem, 2 — macronucleus, 3 — ciliary band, 4 — infundibulum, 5 — peristome, 6 — protuberance of the epistomial disc, 8 — peristomial border, 9 — contractile vacuole, 10 — micronucleus, B. specimen with contracted peristome: 11 — peristomial cone (formed by contraction of the peristomial border), 12 — space inside the peristomial cone, 13 — scopula

Apiosoma piscicola Blanchard, 1885 (Fig. 2)

In the USSR this species was found on the body surface and on the gills of the following fish species: *Barbus barbus* (L.), *Carassius carassius* (L.), *Al-*

[1] All geographic names are given according to "The World Atlas" edited by Chief Administration of Geodesy and Cartography under The Council of Ministers of the USSR, 2-nd edition, Moscow, 1967

burnus alburnus (L.), *Leuciscus idus* (L.), *Cyprinus carpio* L., and *Coregonus albula* L. The author has examined the populations from six species of fishes (Table 1).

General morphology of the species. Body very elongated, cup-shaped, with sharply tapered adoral part forming a stem. This stem is not an independent part of the body, no border exists between it and the enlarged part. The stem becomes shorter and thicker during metabolic movements. The epistomial

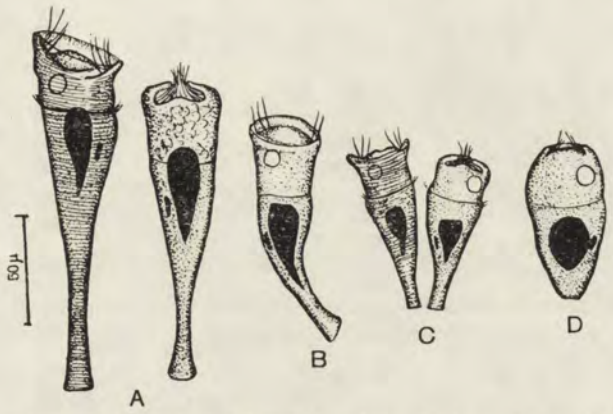

Fig. 2. *Apiosoma piscicola* Blanchard, 1885, A — from body surface of *Leuciscus idus*, B — from body surface of *Rutilus rutilus*, C — from body surface and gills of *Salmo irideus*, D — strongly contracted specimen (fixed, magnification 15 × 40)

disc only sligthly convexed in the centre, surrounded with low protoplasmatic peristomial border. The peristomial cone flattened after contration. The transversal striation of the body well marked. Egg-shaped or triangular macronucleus elongated vertically with its pointed end directed posteriorly. When the body contracts the macronucleus becomes more rounded, widely egg-shaped or almost spherical. Club-shaped or elongated oval micronucleus situated laterally to the macronucleus.

The species as a whole is very variable. Sometimes there were found ciliates with short stem (on *Salmo irideus* G.), sometimes the stem was elongated (on *L. idus* and *C. carpio*). In one case, on *L. idus*, there was found a population of very large ciliates nearly twice exceeding the common dimensions of this species (Table 1).

Apiosoma magna sp. n. (Fig. 3)

This species occurred abundantly on carps, *Cyprinus carpio*, in the pond cultures in the region of Pskov and Svierdlovsk.

The solid body of the ciliate pear- or cup-shaped, gradually tapered into a well marked thick stem. Macronucleus widely egg-shaped, large, oval micronucleus situated laterally to the macronucleus. The epistomial disc only slightly convexed in the centre, surrounded with low, thick peristomial border

Table 1

Dimensions of *Apiosoma piscicola*

Host species and locality	Measurements of the body		Measurements of macronucleus		Measurements of micronucleus	
	length	width	length	width	length	width
Cyprinus carpio						
valley of the river Vachš, river Don	39.6—86.4*	10.8—36.0	5.4—25.2	5.4—14.4	2.3—6.3	1.8—4.3
Rutilus rutilus	55.0±2.6—	20.7±0.8—	14.0±0.6—	9.0±0.4—	3.0±0.14	3.6±0.14
Leningrad region	—61.0±1.8**	—27.0±1.0	—18.1±0.9	—12.6±0.5		
Salmo irideus						
Leningrad Region	43.2—79.2	12.6—25.2	10.8—25.2	6.5—14.5	3.5—6.5	1.0—2.1
Leuciscus idus						
river Ural	57.2±0.9—	18.4±0.4—	15.3±0.3—	8.6±0.2—		
L. leuciscus	—62.0±1.0	—20.9±0.4	—17.6±0.3	—11.4±0.2		
river Kama						
L. idus	57.2—129.6	18.0—54.0	14.4—32.4	7.2—23.4	6.5—7.9	1.8—2.5
Leningrad Region	90.0±3.8	27.0±2.9	20.0±1.8	11.0±1.4	7.2±0.2	2.4±0.3
Various hosts (after Timofeew in Shulman)	23—75	12—33	6—25	6—14	1—4	1—1.5

* — range of dimensions, ** — range of mean values

Table 2

Dimensions of *Apiosoma magna* sp. n.

Host species and locality	Measurements of the body		Measurements of macronucleus		Measurements of micronucleus	
	length	width	length	width	length	width
Cyprinus carpio	61.2—93.6*	21.6—36.0	18.0—28.8	9.0—18.0	3.6—7.2	1.1—2.5
Svierdlovsk Region	77.0±1.9**	29.0±1.4	25.0±1.2	15.0±0.86	5.8	1.8
	50.4—105.1	23.0—54.0	14.4—32.4	10.8—25.2	3.6—9.1	2.8—6.5
C. carpio	82.0±1.3—	36.0±0.8—	21.8±0.58—	16.6±0.32—	5.2±0.3	4.3±0.14
Pskov Region	—89.0±1.8	—37.8±0.9	—26.6±0.43	—17.6±0.54		

* — range of dimensions, ** — mean or range of mean values

forming distinct spiral. The specimens with contracted peristome were never observed in spite of the great number of specimens examined (above 200). The transversal stration of the pellicle only slightly marked. Measurements in Table 2.

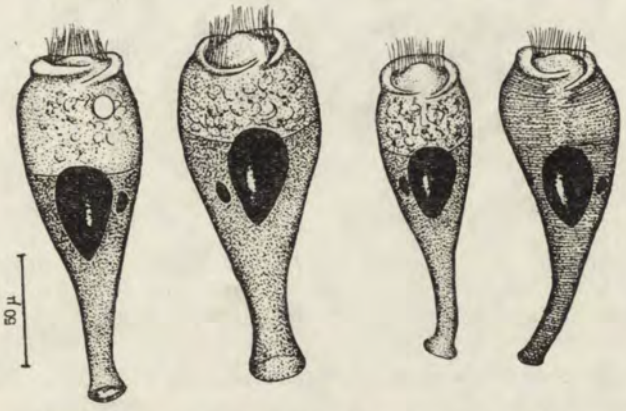

Fig. 3. *Apiosoma magna* sp. n. (fixed, magnification 15 × 40)

This new species bears the most resemblance to *A. piscicola*. There exists in the literature some reports on the incidence of the large ciliates, similar to *A. piscicola*, on the carps (Lom, Rasmashkin). Besides their greater dimensions the ciliates from carps differ from *A. piscicola* by their more solid and rounded body, thicker stem, thick and low peristomial border and more rounded nuclei. By its dimensions *A. magna* sp. n. shows the most similarity to *A. giganthea* (Kandiloff, 1964) found on *Rutilus rutilus caspicus* m. *curensis* Berg from the river Kura (Kandiloff 1964). But the conical body shape with no marked stem, and proportionally small and regularly oval macronucleus in *A. giganthea* distinguish both these species well.

Apiosoma amoeba Grenfell, 1887 (Fig. 4)

This species was found by Timofeew on *Rutilus rutilus, Gasterosteus aculeatus* L. and *Pungitius pungitius* L. in the outlet of Neva. The present author found this species twice (Table 3).

The body conical in shape, sharply tapered towards the scopula, becomes cup-like after contraction. The transversal stration well marked. The epistomial disc large, surrounded with low peristomial border. Well marked protuberance occurs in the centre of the disc. Large macronucleus oval or somewhat triangular in shape. The micronucleus polimorphic, its shape depends upon its position: it is spherical when placed at the anterior part of the macronucleus and oval or club-shaped when it lies laterally or close to the posterior border of the macronucleus. Sometimes it lies so close that it touches the macronucleus or is even squeezed into it. Measurements in Table 3.

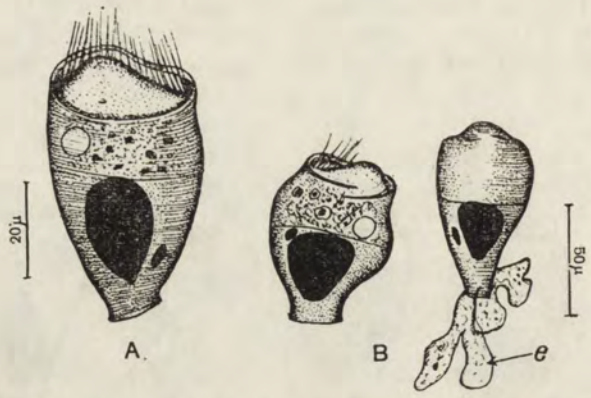

Fig. 4. *Apiosoma amoeba* Grenfell, 1887, A — magnification 10 × 90, B — magnification 15 × 40, *e* — fragment of host epithelium teared out when the infusorian was removed (fixed)

Apiosoma campanulata Timofeew, 1962 (Fig. 5)

It was found by Timofeew on *Perca fluviatilis* L. and *Lucioperca lucioperca* (L.) in the outlet of Neva, and by Lubarskaja in the river Volga. The author's materials were collected from various reservoirs.

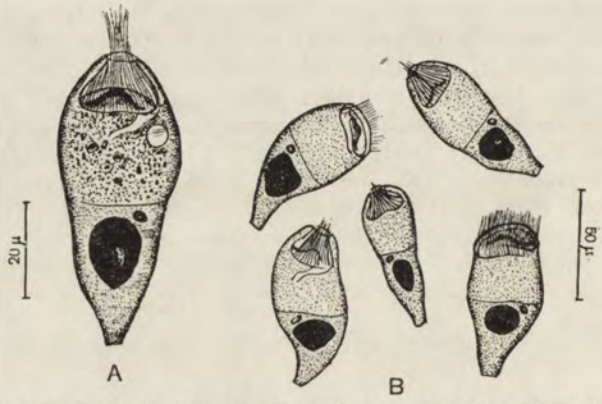

Fig. 5. *Apiosoma campanulata* Timofeew, 1962, A — magnification 10 × 90, B — magnification 15 × 40 (fixed), C — magnification 15 × 40 (living specimens)

The body is pear- or bell-shaped, sharply tapered towards small scopula. The transversal striation of the pellicle feebly developed. The epistomial disc strongly convexed in the centre (in form of a protuberance). The peristomial border moderately high when the peristome is open, but it becomes longer and forms a high cone after contraction. On the preparations the internal cavity of this cone has the shape of an equilateral triangle. This is the characteristic feature of the species. Macronucleus round or egg-shaped. Round or oval micronucleus is situated laterally, near to the anterior border of the macronucleus. The dimensions of the ciliates are given in Table 4.

Table 3

Dimensions of *Apiosoma amoeba*

Host species and locality	Measurements of the body		Measurements of macronucleus		Measurements of micronucleus	
	length	width	length	width	length	width
Leuciscus idus Leningrad Region	28.8—55.8* 41.0±0.9**	14.4—36.0 24.5±0.7	7.2—21.6 15.1±0.5	7.2—23.4 13.3±0.5	1.8—5.4	1.8—3.2
Ctenopharyngodon idella Rostov Region	32.4—54.0 43.1±1.0	18.0—32.4 24.8±0.7	7.2—18.0 11.8±0.4	5.4—14.5 10.1±0.3	2.2—3.6	1.8—2.9
Various hosts (after Timofeew in Shulman)	21—80	12—33	7—28	7—21	1—4	1—3

* — range of dimensions, ** — mean value

Table 4

Dimensions of *Apiosoma campanulata*

Host species and locality	Measurements of the body		Measurements of macronucleus		Measurements of micronucleus	
	length	width	length	width	length	width
Perca fluviatilis Regions of Leningrad and Pskov, rivers Kama and Ural	28.8—67.6* 34.2±0.7— —43.9±0.7**	12.6—28.8 16.6±0.4— —23.7±0.5	5.4—17.9 8.2±0.3— —11.9±0.3	5.4—18.0 8.6±0.5— —11.9±0.25	1.8—3.6	1.0—3.6
Esox lucius rivers Kama and Ural	21.6—61.2 34.2±0.7— —40.0±1.8	12.6—29.0 17.6±0.5— —22.0±0.8	5.4—14.4 9.0±0.2— —9.4±0.3	6.5—18.0 9.4±0.4— —13.0±0.3	1.8—2.9	1.8—2.5
Acerina cernua river Ural, lake Seliger	25.2—57.6 38.9±0.7— —39.2±0.9	12.6—32.4 16.6±0.4— —23.7±0.5	5.4—16.2 10.1±0.4— —11.5±0.5	7.2—14.5 9.8±0.6— —13.7±0.3	1.8—3.6	1.8—3.6
Various hosts (after Timofeew in Shulman)	21—48	13—20	4—18	6—13	1	1

* — range of dimensions, ** — range of mean values

Apiosoma shulmani Kashkowskij, 1965 (Fig. 6)

Kashkowskij found this species on *Lota lota* in Iriklinskij reservoir (the river Ural). The author's materials were gathered from the same host species from Iriklinskij reservoir, from the lake Vrevo (region of Leningrad) and, in a small quantity, from the lake Seliger (region of Kalinin).

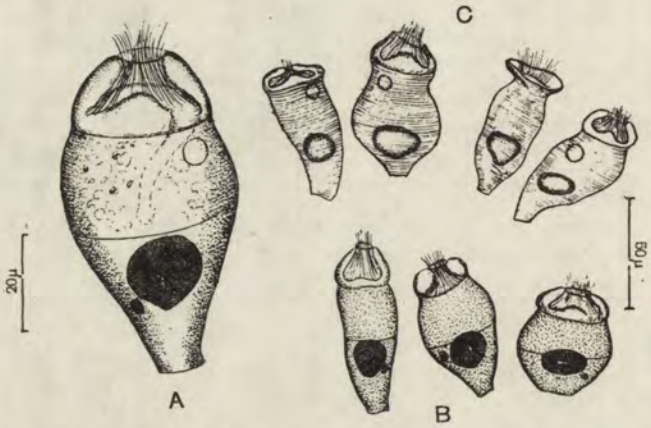

Fig. 6. *Apiosoma shulmani* Kashkowskij, 1965, A — magnification 10 × 90, B — magnification 15 × 40 (fixed), C — magnification 15 × 40 (living specimens)

The body shape is in form of an amphora but it is very changeable due to great inclination to metabolic movements. The transversal striation well marked. The peristomial border well developed, high. In living specimens it may stand upright or be folded at the sides, but during fixation it becomes distinctly thicker forming a thick border surrounding the epistomial disc. The macronucleus is rounded but its shape changes simultaneously with the change of the body shape. Round micronucleus is situated laterally or nearer to the posterior border of the macronucleus. The ciliates collected in the lake Vrevo were distinctly smaller than those from Iriklinskij reservoir. Measurements in Table 5.

Apiosoma carpelli sp. n. (Fig. 7)

This species was found on the carps, *Cyprinus carpio,* from two reservoirs in the region of Leningrad and in the Latvian Republic of the Soviet Union as well as on *Cobitis taenia* L. in the region of Leningrad.

The body conical in shape tapered towards the scopula. The scopula is distinctly differentiated from the body by a slight narrowing. The transversal striation of the pellicle well marked. The epistomial disc without visible protuberance, the peristomial border not very high. In the specimens with contracted peristome the anterior part of the body is flattened. The macronucleus oval or egg-shaped. The position of the micronucleus is variable: it may occur in front of the macronucleus, or laterally to its anterior border. Its shape is connected with its position; it is oval in lateral and club-shaped in frontal position. Measurements in Table 6.

Table 5

Dimensions of *Apiosoma shulmani*

Host species and locality	Measurements of the body		Measurements of macronucleus		Measurements of micronucleus	
	length	width	length	width	length	width
Lota lota Leningrad Region	22.8—46.8* 38.9±0.7**	13.7—28.8 22.0±0.7	5.4—10.1 7.2±0.14	6.1—12.6 10.0±0.3	1.8—3.6 2.7	1.8—2.8 2.3
L. lota river Ural	43.2—62.0 58.0±1.0	18.0—36.0 26.3±0.8	7.2—17.0 11.0±0.5	7.2—18.0 13.3±0.7	1.8—2.8 2.5	2.4—3.0 1.8

* — range of dimensions, ** — mean value

Table 6

Comparison of dimensions of *Apiosoma carpelli* sp. n. and *A. minuta*

Species of parasite and host, locality	Measurements of the body		Measurements of macronucleus		Measurements of micronucleus	
	length	width	length	width	length	width
Apiosoma carpelli *on Cyprinus carpio* Leningrad Region, Latvian SSR	20.6—52.2* 35.6±0.6— —41.0±0.7**	9.0—21.0 14.0±0.25— —15.2±0.3	5.4—11.5 7.9±0.2— —9.0±0.18	4.3—10.8 6.9±0.14— —7.6±0.2	2.0—5.4 3.0±0.07— —7.6±0.14	0.7—2.2 1.4±0.07— —1.7±0.07
Apiosoma carpelli *on Cobitis taenia* Leningrad Region	25.2—54.0 37.8±0.8	10.1—21.6 16.2±0.5	6.5—12.6 9.2±0.3	3.6—10.8 7.0±0.25	0.7—3.6 2.3±0.2	0.7—2.2 1.9±0.1
Apiosoma minuta (after Chen-Chi-lu)	20—29 24.6	9.2—16.9 12.2	—	—	—	—
Apiosoma minuta (after Timofeew in Shulman)	13.7—26.2 16.1	4.9—12.2 8.2	3.7—10.8 8.6	3.7—7.5 6.5	1.0	1.0

* — range of dimensions, ** — mean or range of mean values

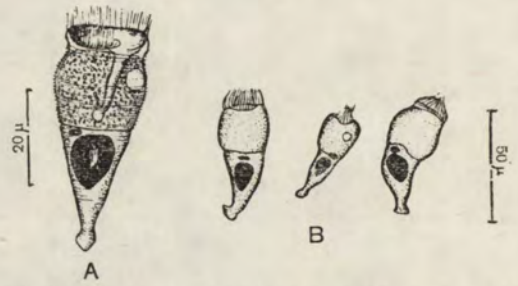

Fig. 7. *Apiosoma carpelli* sp. n., A — magnification 10×90, B — magnification 15× × 40 (fixed)

Taking into account the dimensions this species mostly resembles *A. campanulata* but it differs from it by the lack of the high peristomial cone characteristic of *A. campanulata*. The shape and situation of the nuclei are similar to those in *A. minuta* Chen, 1961 and its subspecies found by Timofeew 1962 on fishes from the river Amur (due to this similarity the species described was primarily determined as *A. minuta* — Banina 1966). But it differs from *A. minuta* by its greater dimensions (Table 6). From both mentioned species *A. carpelli* sp. n. differs by the shape and variability in the position of the micronucleus.

Apiosoma cryptomicronucleata sp. n. (Fig. 8)

This species was found in great quantity on *Gasterosteus aculeatus* and *Pungitius pungitius* in the environs of Leningrad.

The body shape of living specimens is conical, tapered towards the scopula, but it may become shorter and rounded depending on contraction. The macronucleus egg-shaped or spherical. The transversal striation well marked. The body shape in fixed specimens is usually the same as in living ones but the

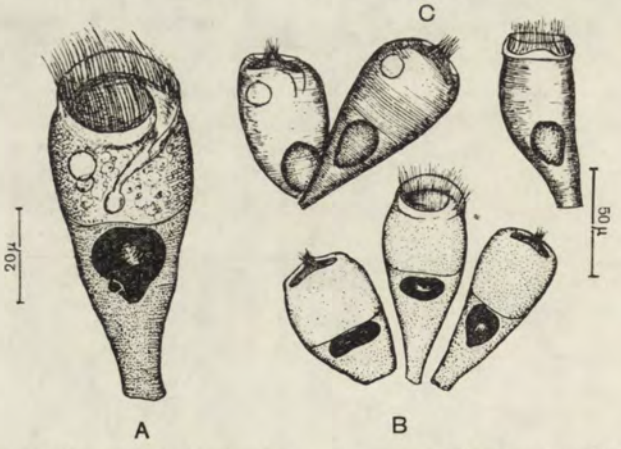

Fig. 8. *Apiosoma cryptomicronucleata* sp. n., A — magnification 10×90, B — magnification 15×40 (fixed), C — magnification 15×40 (living specimens)

shape of the macronucleus may change under fixation; it becomes shorter and wider until it is transversely elongated. The peristomial border not very high; it lies horizontally when the peristome contracts, so the peristomial cone becomes flat. The epistomial disc only slightly convexed in the centre. Micronucleus deeply squeezed into the macronucleus, so it is hardly discernible. There was no possibility to measure it. Sometimes one end of the micronucleus projects out of the lower part of the macronucleus, and sometimes there is visible only a depression in shape of an oblique groove. Measurements in Table 7. The dimensions of the ciliates from two populations examined were somewhat different.

Table 7

Dimensions of *Apiosoma cryptomicronucleata* sp. n.

Host	Measurements of the body		Measurements of macronucleus	
	length	width	length	width
Pungitius pungitius	36.0—79.6* 60.0± 1.3**	16.2—28.8 24.5± 0.5	9.0—21.6 13.9± 0.3	7.2—16.2 13.1±0.55
Gasterosteus aculeatus	36.0—68.4 49.0± 1.2	21.0—36.0 27.4±0.75	7.2—18.0 12.6± 0.5	9.0—21.6 14.8± 0.5

* — range of dimensions, ** — mean value

Such structure of the nuclear apparatus resembles those described by Timofeew in *A. doliaris* and *A. nasalis*. But *A. cryptomicronucleata* sp. n. differs from them both by the body shape. It is conical in the species described whereas cylindrical and barrel-shaped in both mentioned above. On the other hand the shape and body dimensions are similar to *A. conica*, described also by Timofeew, but the nuclear apparatus is quite different (the micronucleus lies laterally to the macronucleus in *A. conica*).

The differences mentioned above enable us to distinguish the species described as a new one.

Apiosoma baueri Kashkowskij, 1965 (Fig. 9)

This ciliate was found by Kashkowskij on the body surface of *Perca fluviatilis* from Iriklinskij reservoir (the river Ural). The author's materials were collected from the body surface of *Leucaspius delineatus* in the region of Pskov and from the gills of *Esox lucius* in the region of Leningrad.

Barrel-shaped body, large, round macronucleus and oval micronucleus situated laterally, near to the posterior border or even in front of the macronucleus are characteristic features distinguishing this species from the others. The peristomial border not very high, the epistomial disc flat, without any protuberance. Measurements in Table 8.

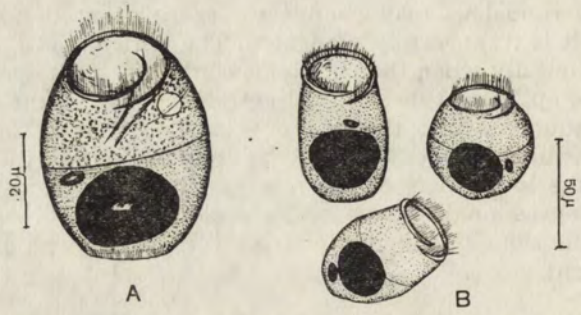

Fig. 9. *Apiosoma baueri* Kashkowskij, 1965, A — magnification 10×90, B — magnification 15×40 (fixed), C — magnification 15×40 (living specimens)

Table 8

Dimensions of *Apiosoma baueri*

Host	Measurements of the body		Measurements of macronucleus		Measurements of micronucleus	
	length	width	length	width	length	width
Leucaspius delineatus	28.8—57.6*	19.8—39.6	9.0—25.2	14.4—21.6	3.6—6.5	2.2—3.6
	44.3± 0.8**	28.8± 0.6	16.9	17.6	4.7	3.0
Esox lucius	30.6—57.6	19.8—39.6	9.0—21.6	10.8—21.6	1.8—3.6	1.8—4.3
	42.5± 0.9	29.2± 0.5	15.5± 0.4	14.2±0.36	3.2±0.1	3.1±0.1

* — range of dimensions, ** — mean value

Apiosoma megamicronucleata Timofeew, 1962 (Fig. 10)

The species was found by Timofeew on *Lota lota* from the river Amur and soon after it was reported to have been found in lake Bajkal (Zajka 1965). The characteristic structure of the nuclear apparatus distinguishes this species from the others.

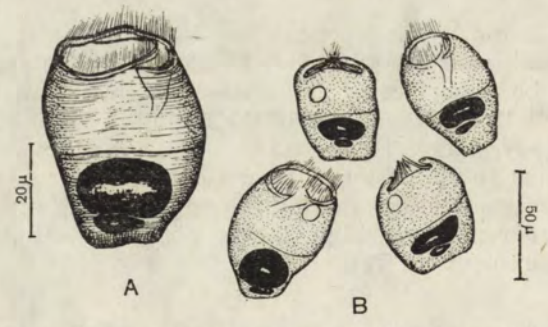

Fig. 10. *Apiosoma megamicronucleata* Timofeew, 1962, (fixed)

The body is barrel- or cup-shaped with wide scopula. Large, bean-shaped macronucleus situated transversely to the body axis. Large, transversely elongated micronucleus is situated in the concavity at the back of the macronucleus. The epistomial disc nearly flat, the peristomial border low; it lies nearly horizontally when contracted. The anterior part of the body with contracted peristome only slightly convexed. The transversal striation feebly developed. Measurements in Table 9.

This ciliate was found also on *L. lota* and *R. rutilus* in Karelia (Rumjantzev 1966). The materials of the present author were collected from several reservoirs from *L. lota* and, in a small quantity, from *E. lucius* and *R. rutilus*.

Apiosoma minimicronucleata sp. n. Fig. 11

This species of *Apiosoma* was found on the gills of perch *Perca fluviatilis* in Iriklinskij reservoir (the river Ural).

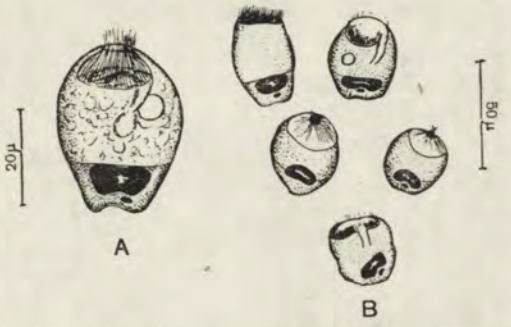

Fig. 11. *Apiosoma minimicronucleata* sp. n., A — magnification 10×90, B — magnification 15×40 (fixed)

This barrel-shaped ciliate resembles *A. megamicronucleata* by its shape, but differs from the latter by smaller dimensions. The transversal striation feebly developed and also the ciliary band. The peristomial border not very high; when contracted it forms wide, flattened peristomial cone. The cilia, inserted inside the peristomial cone, are visible through the transparent wall and give it radially striated appearance. Similarly as in *A. megamicronucleata*, the macronucleus is transversely elongated, often bean-shaped. The micronucleus is situated at the back of the macronucleus in its concavity. It is also transversely elongated but very small and inconspicuous. The name *A. minimicronucleata* is proposed on account of very small dimensions of the micronucleus. Measurements in Table 10.

The comparative studies of several species of *Apiosoma* give a basis for appretiation of the specific characters in this group of ciliates together with some practical conclusions dealing with the preparation of the material for investigations.

From the point of view of specific diagnosis, the shape and situation of the nuclei inside the ciliate body appears to be the most stable character. The acquisition of this character as a main principle for determination of the

Table 9

Dimensions of *Apiosoma megamicronucleata*

Host species and locality	Measurement of the body		Measurement of macronucleus		Measurement of micronucleus	
	length	width	length	width	length	width
Lota lota Regions and Pskov and Leningrad, rivers Kama and Ural	28.8—50.4* 37.0±0.6— —41.8±0.6**	14.6—36.0 24.8±0.4— —29.2±0.6	4.7—13.7 7.3±0.2— —10.3±0.2	7.2—25.0 12.4±0.2— —19.6±0.5	2.5—7.2 3.2±0.1— —5.0±0.14	3.6—10.8 5.6±0.15— —7.2±0.3
Esox lucius Pskov Region	32.4—61.2 44.0±1.0	24.1—50.0 36.0±0.8	7.2—14.4 10.6±0.4	14.4—28.8	3.6—6.5 5.0±0.14	5.4—14.4 8.0±0.5
Lota lota (after Timofeew in Shulman)	30.0—42.0	18.0—30.0	3.0—9.0	9.0—12.0	3.0—4.5	3.0—7.5

* — range of dimensions, ** — mean or range of mean values

Table 10

Dimensions of *Apiosoma minimicronucleata* sp. n.

Host species and locality	Measurements of the body		Measurements of macronucleus		Measurements of micronucleus	
	length	width	length	width	length	width
Perca fluviatilis Iriklinskij reservoir on river Ural	16.2—43.2* 26.0±0.7**	16.3—32.4 24.9±0.43	3.6—9.0 6.4±0.1	7.2—14.4 12.4±0.3	0.42—0.66	1.8—2.2

* — range of dimensions, ** — mean value

species in the key: "Opredelitel parazitov presnovodnych ryb SSSR" (1962) must be regarded as methodically correct.

It is more difficult to estimate the body shape subjected to great variations and to describe it correctly. The resemblance of this character in a series of species must be taken into account. Basing on the body shape three groups of the species may be distinguished in the *Apiosoma,* namely: the ciliates with cup-shaped body provided with more or less elongated stem (*A. piscicola, A. magna*), the ciliates with conical, bell- and pear-shaped body sharply tapered towards the small scopula but without differentiated stem (*A. campanulata, A. amoeba, A. shulmani* etc.) and the ciliates with barrel- or cup--shaped body with wide scopula (*A. megamicronucleata, A. baueri, A. minimicronucleata*). The appretiation of this feature must be based on examination of large populations because only a survey of a series of specimens enable us to choose the most characteristic body shape of the species examined.

The third feature, that has been overlooked till now and not mentioned in the descriptions of the species, is the shape of the peristomial disc and the peristomial border as well as the shape of the peristomial cone formed during contraction of the peristome. These structures are peculiar for several species (*A. campanulata, A. shulmani* and the others) and they are rather stable in contrast to other features.

In spite of great variability, the body dimensions have defined limits and the ranges of statistically counted mean values are characteristic of the majority of the species. The dimensions of the nuclei are variable to a lesser degree. Thus the dimensions should be included in the specific diagnosis. In each case statistically significant number of specimens should be measured.

As taxonomic characters of *Apiosoma,* Lom 1966 recommends the number of circular argentophilic annuli on the pellicle, revealed by silver impregnation after Klein, and the buccal infraciliature. It seems that these characters may be usefull mainly in a case when there are no other, more conspicuous, morphological differences between the species.

Finally several methodical recommendations. When studying this group of parasites the Soviet authors have used the same methods as for other groups of *Urceolariidae,* namely: the smears fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin and silver impregnated dry smears. This last method is widely accepted abroad, especially by Lom. The localization of *Apiosoma* on its host body is identical with that of *Trichodina* (body surface, gills and nasal ducts), so both these parasites may be found on the same smears. But the body structure of *Apiosoma,* as well as of other sessiline peritrichs, differs from that of *Trichodina* by a lack of the skeletal ring which is the main taxonomic character in the latter.

As for the treatment of the material for determination care must be taken to prevent the deformations of the ciliate body during fixation. For this purpose the Schaudinn's fixative is recommended as giving the smallest changes in the body shape. The smears must be fixed quickly, immediately after they have been prepared to prevent drying.

The comparison of the body dimensions of the living specimens with those of the ciliates fixed in Schaudinn's fluid showed that the linear body dimensions diminished about 1/4—1/5 of their initial length, but the proportions of the body width to length were almost constant in each species examined (Table 11). These results give the possibility to measure fixed

Table 11

Comparison of the body proportions of living and fixed specimens

Species of parasite	Mean body width to length proportion	
	living	fixed
A. carpelli sp. n.	0.52	0.48
A. piscicola (with short stem)	0.39	0.39
A. piscicola (large form)	0.29	0.30
A. amoeba	0.63	0.59
A. megamicronucleata	0.70	0.73

ciliates instead the living ones and the probability of preserving the right proportions of the body is high.

Contrary to that the method of Klein, applied for dry smears, causes the deformations of the ciliate body when they dry up, so it may be used only together with Schaudinn's fixated smears or with examination of the living specimens.

But in each case the examination of large population is recommended because single specimens may differ greatly from the typical forms. It is usefull to draw the outline of a series of the most typical representatives of the population. This method makes the survey of the material easier and allows to choose the most characteristic features.

The author wishes to express her gratitude to A. M. Lopuchina, J. A. Strelkov, O. N. Junčis, W. W. Kashkovskij, and U. D. Džalilov from the Laboratory of Fish Diseases, State Institute of Freshwater Fisheries, for their help in collecting the materials.

Summary

The studies were carried on the ciliates of the genus *Apiosoma (Peritricha Sessilia, Scyphidiidae)* occurring on fresh-water fishes in the European part of USSR. Great variability of these ciliates causes some difficulties in distinction of the species and in description of them. The author attempted to distinquish the most conspicuous taxonomical features, to precise the descriptions of the species and to give some methodical recommendations for collecting and treatment of the material.

Out of ten species examined four appeared to be new, namely *Apiosoma magna, A. carpelli, A. cryptomicronucleata,* and *A. minimicronucleata.*

It must be admitted that the most stable specific characters of the *Apiosoma* are succesively: the shape and situation of the nuclei, the shape of the body, and the dimensions of the body and of the nuclei. The structure of the peristome is also a good taxonomic feature (including the character of the peristomial disc, the shape and height of the peristomial border, the shape of the cone formed during contraction of the peristome, and the shape of the conical space).

Examination of the mass material is recommended in order to obtain the

right figure of the body shape and correct dimensions. The data concerning the body dimensions should be worked out with the aid of statistical methods.

РЕЗЮМЕ

Статья посвящена изучению фауны инфузорий рода Apiosoma (*Peritricha, Sessilia*, семейство *Scyphidiidae*), обитающих на пресноводных рыбах Европейской части СССР. Большая изменчивость этих инфузорий затрудняет их описание и установление видовых различий. Автор стремился выделить наиболее четкие систематические признаки, утончить описания видов и дать методические рекомендации для сбора и обработки материала.

Из десяти изученных автором видов инфузорий четыре *Apiosoma magna. A. carpelli, A. cryptomicronucleata* и *A. megamicronucleata* оказались новыми. В роду *Apiosoma* можно различить виды, обладающие более широким диапазоном изменчивости (*A. piscicola, A. amoeba*) и виды с более стабильными признаками (*A. campanulata, A. megamicronucleata, A. shulmani* и нек. другие).

Наиболее стабильными видовыми празнаками у *Apiosoma* следует считать форму и взаимное расположение ядер, затем форму тела и, наконец, размеры тела и ядер. Хорошие систематические различия наблюдаются также в строении перистома (характер перистомального поля, форма и высота перистомальной каймы и конуса, образующегося при смыкании ее краев, форма внутреннего пространства перистомального конуса).

Для получения правильного представления о форме и размерах тела инфузорий изучение рекомендуется вести на массовом материале, данные о размерах обрабатывать статистическим методом. Размеры инфузорий в разных популяциях одного и того же вида в некоторых случаях могут колебаться, вследствие чего разницу в размерах, при отсутствии других различий, нельзя считать достаточным основанием для систематического разделения форм. Фиксацию инфузорий для приготовления препаратов следует производить быстро, не допуская подсыхания мазка, так как это приводит к резкой деформации тела.

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On gregarine parasites of *Coccinellidae* in California, USA

O gregarynach pasożytujących w biedronkach (*Coccinellidae*) w Kalifornii, USA

A great majority of lady-bird beetles are beneficial insects and play an important role in the natural control of noxious insects (Hodek 1967). Only some species e.g. *Epilachna varivestis* Muls. are known as pests of cultivated plants. Parasites and pathogens of *Coccinellidae* have attracted attention of many entomologists as they can be used to control noxious species. In case of beneficial *Coccinellidae* some methods of protecting them against parasites should be worked out. This is especially important in case of *Coccinellidae* which are reared in mass in insectaries in order to release them in the field against various pests. Pathogenic organisms can make it completely impossible to rear *Coccinellidae* in mass.

In 1959, while working as a fellow of the Rockefeller Foundation at the Division of Invertebrate Pathology, University of California, Berkeley, I had the opportunity to study protozoan parasites of *Coccinellidae*. Results of studies on the microsporidian parasite of *Hippodamia convergens* Guerin are reported elsewhere (Lipa and Steinhaus 1959, 1962). Here results of studies on gregarine parasites are included.

Materials and methods

Specimens of various species of *Coccinellidae* were collected by the author in June, July and August of 1959 in various localities in California. Some insects were kindly supplied by Drs. K. M. Hagen and C. Gonzales of the University of California.

Insects were anastethized, dissected in saline and contents of their gut were examined under the microscope. Gregarines were measured, drawn or photographed and eventually used to prepare permanent microscopic preparations stained with 1% Giemsa's solution for 20 hours.

Abbreviations used in text and in tables:

Prim. — primite
Sat. — satellite
LP — length of protomerite
LD — length of deutomerite
WP — width of protomerite
WD — width of deutomerite
TL — total length of gamont
TLA — total length of association

Results

During this study five species of *Coccinellidae*, commonly occurring during the summer months in California were examined, and four of them were found to be infected with gregarines (Table 1).

Table 1

A list of examined *Coccinellidae* and being parasitized by gregarines in California

Host insect	Gregarine species
Coccinella californica	*Gregarina katherina* Watson *Gregarina californica* sp. n.
Coccinella trifasciata	*Gregarina barbarara* Watson *Gregarina fragilis* Watson *Gregarina katherina* Watson
Hippodamia convergens	*Gregarina barbarara* Watson
Hippodamia sinuata	*Gregarina barbarara* Watson
Hippodamia quinquesignata punctulata	not parasitized

1. *Gregarina barbarara* Watson, 1916

Host insects: *Coccinella trifasciata* L., *Hippodamia convergens* Guerin, *H. sinuata* M.
Habitat: intestine.
Locality record: Berkeley, July 1959.

Morphology: Gamonts in associations, oval (Figs. 1—3; Pl. I 1,2). Maximum length 141 μ, maximum width 78 μ. Primite: ratio LP:TL = 1:4.2—6.1; ratio WP:WD = 1:1.5—2.6. Satellite: ratio LP:TL = 1:7.3—12; ratio WP:WD = 1:1.1—2.3 (Table 2).

Primite: Protomerite subglobular, wider than long. Septum and constriction well seen. Endoplasm lightly granular and frequently transparent. Deutomerite elipsoidal or slightly elongated. Endoplasm dark and not translucent. The nucleus about 15 μ in diameter with one large karyosome.

Satellite: The shape of the satellites is quite different from that of the primite. Protomerite flattened (Figs 1—3, Plate I 1) and frequently no constriction present at the septum. The deutomerite widest a little behind the septum and slighty tempers toward the end. In some associations two satellites attached to a single primite were observed (Pl. I 2).

Cysts and spores: Cysts are oval, up to 200 μ in diameter, with several sporeducts. Spores are spindle-shaped 10—12 μ long.

Parasitization: *Gregarina barbarara* was observed in *Coccinella trifasciata*, *Hippodamia convergens* and *H. sinuata*, but no differences were observed in the intensity of infection of these three species. In some host insects up to 100 gregarines were observed.

Table 2

Measurements of gamonts of *Gregarina barbarara* Watson (in microns)

	LP	LD	WP	WD	TL	TLA	Ratio	
							PL:TL	WP:WD
Prim.	25	92	33	61	117	236	1:4.7	1:1.8
Sat.	12	107	47	66	119		1:9.6	1:1.3
Prim.	22	119	33	59	141	254	1:6.1	1:1.8
Sat.	12	100	45	66	112		1:9.2	1:1.4
Prim.	25	82	35	63	107	223	1:4.3	1:1.8
Sat.	14	102	45	70	116		1:8.1	1:1.6
Prim.	25	90	33	74	115	250	1:4.6	1:2.2
Sat.	12	123	53	66	135		1:11	1:2.3
Prim.	27	86	37	66	113	207	1:4.2	1:1.5
Sat.	8	86	41	49	94		1:11.5	1:1.2
Prim.	24	86	33	66	110	209	1:4.5	1:2
Sat.	8	90	49	49	98		1:12	1:1
Prim.	18	76	25	37	94	184	1:5.1	1:1.5
Sat.	12	78	22	33	90		1:7.3	1:1.4
Prim.	18	82	25	49	100	202	1:5.4	1:2
Sat.	12	90	28	37	102		1:8.3	1:1.2
Prim.	16	68	22	35	86	180	1:5.2	1:1.5
Sat.	12	82	22	31	94		1:7.6	1:1.3
Prim.	18	82	20	45	100	219	1:5.4	1:1.5
Sat.	12	107	36	37	119		1:9.6	1:1

Taxonomic position: Size and shape of the investigated gregarine closely fit the characteristic of *Gregarina barbarara* Watson, 1916 described from *Coccinella* sp. Watson 1916 mentioned that the parasite is practically transparent while in my material some gamonts had a granular endoplasm and were well seen.

As the studied gregarine closely resembles the species described by Watson 1916 I identify it as *Gregarina barbarara* Watson.

Gregarina barbarara Watson sensu Foerster, 1938, reported in Europe in *Coccinella septempunctata* L. and *Hippodamia tredecimpunctata* L., was considered by Lipa 1967 to be the synonym of *Gregarina coccinellae* Lipa.

Distribution: USA (Kansas, California).

2. *Gregarina californica* sp. n.

Host insect: *Coccinella californica* Mann.
Habitat: Intestine.
Locality record: Berkeley and vicinity, July to August, 1959.

Morphology: Gamonts in associations, elipsoidal (Pl. I 3). Maximum length 184 μ, maximum width 127 μ. Primite: ratio LP:TL = 1:5.1—7.4; ratio WP:WD = 1:2.1—2.8. Satellite: ratio LP:TL = 1:6.8—11.2, ratio WP:WD = = 1:1.5—1.7 (Table 3).

Table 3

Measurements of gamonts of *Gregarina californica* sp. n. (in microns)

	LP	LD	WP	WD	TL	TLA	Ratio	
							LP:TL	WP:WD
Prim.	25	119	29	78	144	295	1:5.8	1:2.7
Sat.	16	127	41	70	141		1:8.6	1:1.7
Prim.	27	137	35	98	164	319	1:6.1	1:2.8
Sat.	16	139	53	90	156		1:9.5	1:1.6
Prim.	25	159	61	135	183	352	1:7.4	1:2.1
Sat.	25	144	70	111	169		1:6.8	1:1.5
Prim.	33	135	55	118	168	352	1:5.1	1:2.1
Sat.	16	168	82	127	184		1:11.2	1:1.5

Primite: Protomerite subglobular, wider than long. Constriction and septum well seen. Endoplasm granular and dark. Deutomerite elipsoidal or oval. Endoplasm of deutomerite granular and dark. Nucleus about 15 μ in diameter, well seen as a white spot against the dark endoplasm (Pl. I 3).

Satellite: The satellite similar to primite except that the protomerite is much shorter than that of primite. Septum and constriction seen. Endoplasm granular and not translucent. Deutomerite elongated cylindrically and widest at the shoulder (Pl. I 3). Endoplasm granular and dark. Nucleus about 20 μ in diameter, well seen as a white spot in the dark endoplasm.

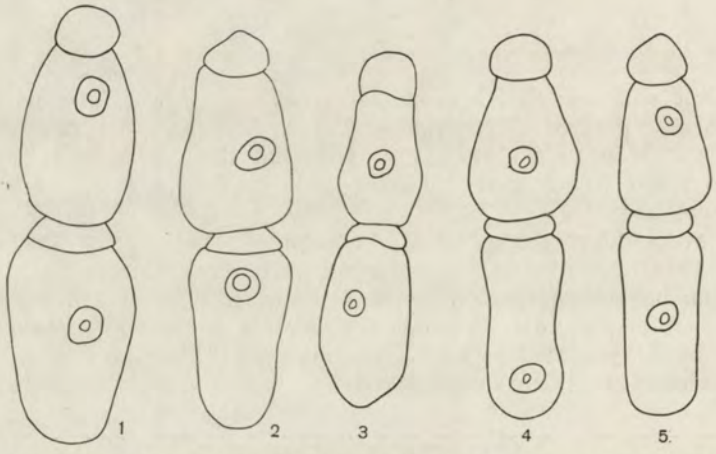

Fig. 1—3. Gamonts of *Gregarina barbarara* Watson from various hosts: 1 — from *Hippodamia sinuata*, 2 — from *Coccinella californica*, 3 — from *Hippodamia convergens*

Figs. 4—5. Gamonts of *Gregarina fragilis* Watson from *Coccinella trifasciata*

Cysts and spores: not observed.

Parasitization: fifteen associations were observed in one adult beetle of *Coccinella californica*.

Taxonomic position: This gregarine greatly differs from other species known in *Coccinellidae*. First of all, it is much larger in size and its nucleus is well seen as a white spot. From *G. barbarara,* which is smaller it differs in the shape of the satellite. From *G. katherina* and *G. fragilis* it differs by a much greater width. Accordingly, I assume it is a new species and propose the name *Gregarina californica* sp. n. for it.

Distribution: USA (California).

3. *Gregarina fragilis* Watson, 1916

Host: *Coccinella trifasciata* L.
Habitat: intestine.
Locality record: Berkeley and vicinity, July to August, 1959.

Morphology: Gamonts in associations, cylindrically elongated (Figs 4, 5). Maximum length 120 μ, maximum width 68 μ. Primite: ratio LP:TL = = 1:3.8—5.9, ratio WP:WD = 1:1.6—2. Satellite: ratio LP:TL = 1:7—9, ratio WP:WD = 1:1.4—4. (Table 4).

Table 4

Measurements of gamonts of *Gregarina fragilis* Watson (in microns)

	LP	LD	WP	WD	TL	TLA	Ratio	
							LP:TL	WP:WD
Prim.	20	74	37	66	94	211	1:4.6	1:1.8
Sat.	18	98	41	49	107		1:5.7	1:1.2
Prim.	18	70	28	57	88	202	1:4.2	1:2
Sat.	12	102	35	41	114		1:9	1:1.2
Prim.	25	74	33	53	99	198	1:4	1:1.6
Sat.	12	88	43	47	100		1:8.2	1:1.1
Prim.	25	70	31	49	94	212	1:3 8	1:1.6
Sat.	12	94	33	39	106		1:8.7	1:1.2
Prim.	22	90	31	49	112	233	1:5	1:1.6
Sat.	16	104	34	45	120		1:7.3	1:1.3
Prim.	18	90	33	68	109	227	1:5.9	1:2
Sat.	16	102	39	57	118		1:7	1:4
Troph.	22	86	29	53	109		1:4.8	1:1.8

Primite: Protomerite triangular wider than long (Figs 4, 5). Endocyte usually granular and not translucent. Deutomerite pearlike, widest at the end of the body. Endoplasm not translucent.

Satellite: The satellite is quite different from the primite. The protomerite is largely flattened. Septum and constriction well seen. Endoplasm translucent. Usually in half the length of the body there is a vide constriction, so the deutomerite is narrowest in the middle region of its length.

Cysts and spores: Cysts oval about 200 μ in diameter. Spores were not seen.

Parasitization: Intensity of infection is not very great and up to 30 associations were seen in the host insect.

Taxonomic position: The investigated gregarine very closely resembles *Gregarina fragilis* Watson, 1916, described from *Coccinella novemnotata*. The measurements of both gregarines are the same and the shape is very similar. However, there is a slight difference in color; Watson 1916, observed only translucent gregarines while in my material they were both dark and translucent. As gregarines observed in *C. trifasciata* are very similar in shape and size to the gregarine described by Watson 1916 I identyfy it as *Gregarina fragilis* Watson.

Distribution: USA (Kansas, California).

4. *Gregarina katherina* Watson, 1916

Host: *Coccinella californica* Mann., *C. trifasciata* L.
Habitat: intestine.
Locality record: Berkeley and vicinity, July 15, 1959.

Morphology: Gamonts elongated occurring in syzygies of two or three in chain (Pl. I 4, 5). The maximum length is 94 μ and maximum width 39 μ. Primite: ratio LP:TL = 1:4.7—6.9; ratio WP:WD = 1:1.3—2.1. Satellite: ratio LP:LD = 1:4.7—14; ratio WP:WD = 1:1.1—7 (Table 5).

Primite: Protomerite subglobular, wider than long. Septum and constriction seen. The endoplasm, homogenous or slightly granular is translucent. Deutomerite cylindrical and its endoplasm is granular. The nucleus has one karyosome.

Table 5

Measurements of gamonts of *Gregarina katherina* Watson (in microns)

	LP	LD	WP	WD	TL	TLA	Ratio	
							LP:TL	WP:WD
Prim.	10	59	16	27	69		1:6.9	1:1.6
Sat. I	4	57	14	25	61	195	1:14	1:1.7
Sat. II	8	57	14	25	65		1:8	1:1.7
Prim.	12	49	17	21	61		1:5	1:1.2
Sat. I	8	47	17	22	55	166	1:6.7	1:1.3
Sat. II	9	41	16	20	50		1:5.5	1:1.2
Prim.	11	45	17	22	56	119	1:5	1:1.3
Sat.	8	55	18	20	63		1:7.7	1:1.1
Prim.	8	37	15	20	45	92	1:5.5	1:1.3
Sat.	6	41	16	18	47		1:7.7	1:1.1
Prim.	8	37	16	20	45	92	1:5.5	1:1.3
Sat.	8	39	16	18	47		1:5.7	1:1.1
Prim.	10	41	18	27	51	125	1:5	1:1.5
Sat.	8	66	20	23	74		1:9	1:1.1
Prim.	20	74	25	39	94	151	1:4.7	1:1.5
Sat.	12	45	25	33	57		1:4.7	1:1.3
Prim.	16	66	25	35	82	141	1:5	1:2.1
Sat.	8	51	16	22	59		1:7.2	1:1.3

Satellite: Its length is equal or shorter than that of primite. Protomerite wider than long. Constriction and septum well seen. Endoplasm slightly granular. Deutomerite cylindrical, sometimes elipsoidal. Endoplasm has a texture as in protomerite.

Parasitization: The intensity of infection is medium, as up to thirty syzygies were observed in the host insects. Some insects were simultaneously infected with *Gregarina katherina* and *Gregarina fragilis*.

Taxonomic position: The investigated gregarine is identified as *Gregarina katherina* Watson, 1916, as it resembles this species by its shape, size, and homogenous and translucent endoplasm. However, I observed syzygies of three sporonts in a chain, the feature that was not reported by Watson 1916.

Lipa 1967 described *Gregarina ruszkowskii* Lipa which in some respects resembles *Gregarina katherina*. It also makes associations of three or even four gamonts but it differs from *G. katherina* by its different shape and width. Gamonts of *G. ruszkowskii* are oval while gamonts of *G. katherina* are elongated and are twice narrower. Also other features e.g. shape and texture indicate that these species are separate and valid.

Foerster 1938 claimed that he recorded *G. katherina* in Europe in *C. septempunctata* and *C. quinquepustulata*. However, as I discussed it elsewhere (Lipa 1967) *Gregarina katherina* Watson sensu Foerster, 1938 should be recognized as *Gregarina ruszkowskii* Lipa, 1967.

Distribution: USA (Kansas and California).

Discussion

Coccinellidae are beneficial insects and frequently used in the biological control of noxious insects (Hodek 1967). Therefore special attention should be given to factors that suppress their occurrence and to methods protecting them against parasites, predators, and pathogens. The last is especially important in case of these *Coccinellidae* that are reared in mass in insectaries with the purpose of using them as biological control agents.

In spite of these reasons little is known on pathogens of *Coccinellidae*. Iperti 1964 and Lipa i Semjanow 1967 have studied in detail various parasites and pathogens of several species of *Coccinellidae*. As far as protozoans are concerned there are few records of gregarines and microsporidian infections among *Coccinellidae* (Watson 1916, Foerster 1938, Dellucchi 1954, Lipa and Steinhaus 1959, 1962; Lipa 1967, 1968, Lipa i Semjanov 1967).

Beside practical aspects of studying the mortality factors of *Coccinellidae* this group of insects may be an useful object of zoogeographical distribution of their parasites.

As far as Europe is concerned protozoan parasites of *Coccinellidae* have a rather wide distribution. Lipa 1967, 1968 and Lipa i Semjanov 1967 recorded *Nosema coccinellae* Lipa and *Gregarina coccinellae* Lipa in Poland and in the Soviet Union. Gregarines of *Coccinellidae* observed in Poland (Lipa 1967) occur in Germany and probably in France.

Foerster 1938 claimed that he had found in Europe two gregarines *Gregarina barbarara* Watson and *G. katherina* Watson that had been described

Table 6

A list of protozoans reported from *Coccinellidae*

Protozoan	Host insect	References
MICROSPORIDIA		
Nosema coccinellae Lipa	*Coccinella semptempunctata* L. *Myrrha octodecimguttata* L.	Lipa 1968, Lipa i Semjanov 1967
Nosema hippodamiae Lipa et Steinhaus	*Hippodamia convergens* Guerin	Lipa and Steinhaus 1959, 1962
Nosema tracheophila Cali et Briggs	*Coccinella septempunctata* L.	Cali and Briggs 1967
GREGARINOMORPHA		
Gregarina barbarara Watson	*Coccinella trifasciata* L. *Coccinella* sp. *Hippodamia sinuata* Muls.	Watson 1916, Lipa, this paper
Gregarina californica Lipa	*Coccinella californica* Mann.	Lipa, this paper
Gregarina coccinellae Lipa	*Coccinella septempunctata* L. *Exochomus quadripustulatus* L. *Hippodamia tredecimpunctata* L. *Myrrha octodecimpunctata* L. *Tytthaspis sedecimpunctata* L.	Lipa 1967, Lipa i Semjanov 1967, Foerster 1938
Gregarina fragilis Watson	*Coccinella trifasciata* L. *Coccinella* sp.	Watson 1916, Lipa, this paper
Gregarina katherina Watson	*Coccinella californica* Mann. *Coccinella trifasciata* L. *Coccinella novemnotata* Herbst.	Watson 1916; Lipa, this paper
Gregarina ruszkowskii Lipa	*Coccinella quinquepunctata* L. *Coccinella quatuordecimguttata* L. *Coccinella septempunctata* L.	Lipa 1967; Foerster 1937
Unidentified gregarines	*Adonia variegata* Goeze *Coccinella septempunctata* L. *Coccinella decempunctata* L. *Synharmonia conglobata* L. *Harmonia quadripunctata* L.	Iperti 1964
Unidentified gregarine	*Pullus impexus* Muls.	Dellucchi 1954

from *Coccinellidae* in the United States (Watson 1916). However, as discussed in this paper and elsewhere (Lipa 1967) gregarines recorded by Foerster were in fact two newly described species *Gregarina coccinellae*

Lipa and *G. ruszkowskii* Lipa. The question, whether gregarine parasites of *Coccinellidae* occurring in Europe, are distributed also in other continents and vice versa is still open. These and other questions should be studied further.

Summary

Five *Coccinellidae* species collected in California, USA, were found to be infected with four species of gregarines. They include: *Gregarina barbarara* Watson in *Coccinella trifasciata* L., *Hippodamia convergens* Guerin, and *H. sinuata* M.; *Gregarina californica* sp. n. in *Coccinella californica* Mann.; *Gregarina fragilis* Watson in *Coccinella trifasciata* L.; *Gregarina katherina* Watson in *Coccinella californica* Mann. and *C. trifasciata* L. The morphology of the parasites was described in detail including measurements of their bodies. The importance of studies on pathogens, parasites and predators of *Coccinellidae* was discussed. A list of protozoan parasites of *Coccinellidae* was given.

STRESZCZENIE

Pięć gatunków biedronek (*Coccinellidae*) występujących w Kalifornii USA było zarażonych przez cztery gatunki gregaryn: *Gregarina barbarara* Watson pasożytowała w *Coccinella trifasciata* L., *Hippodamia convergens* Guerin i *H. sinuata* M.; *Gregarina californica* sp. n w *Coccinella californica* Mann.; *Gregarina fragilis* Watson w *Coccinella trifasciata* L.; *Gregarina katherina* Watson w *Coccinella californica* Mann. i *C. trifasciata* L. Opisano szczegółowo morfologię badanych pasożytów podając jednocześnie ich wymiary. Omówiono znaczenie badań nad patogenami, pasożytami i drapieżcami biedronek (*Coccinellidae*). Podano listę pierwotniaków pasożytujących w *Coccinellidae*.

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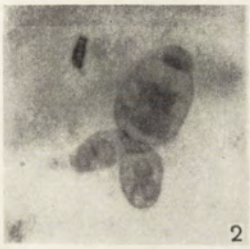
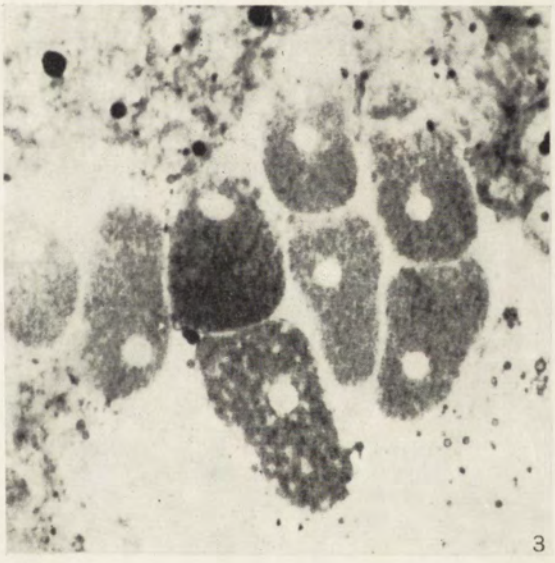
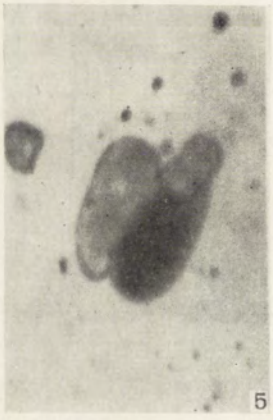
EXPLANATION OF PLATE I

1: Association of gamonts of *Gregarina barbarara* Watson from *Coccinella trifasciata*.
2: Association of *Gregarina barbarara* Watson with two satellites attached to one primite
3: Association of gamonts of *Gregarina californica* sp. n.
4: Association of gamonts of *Gregarina katherina* Watson
5: Two single gamonts of *Gregarina fragilis* Watson







J. J. Lipa auctor phot.

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Some observations on *Nosema heliothidis* Lutz et Splendore, a microsporidian parasite of *Heliothis zea* (Boddie) (*Lepidoptera, Noctuidae*)

Obserwacje nad *Nosema heliothidis* Lutz et Splendore, mikrosporidiowym pasożytem *Heliothis zea* (Boddie) *(Lepidoptera, Noctuidae)*

Until very recently our knowledge of *Nosema heliothidis* Lutz et Splendore was very limited. The original description of this microsporidian given by Lutz und Splendore 1903, 1904 contained only the name of the host insect and the dimensions of spores. While I was conducting these studies at the University of California at Berkeley[1], Kramer 1959 published a full taxonomic description including the well illustrated life cycle of this parasite developing in *Heliothis zea* (Boddie) and *H. virescens* (Fabricius). Kramer left, however, some problems not fully cleared up, in particular, host tissues attacked. Therefore this paper is especially devoted to problems not covered in Kramer's paper.

Materials and methods

In December 1958, Dr. Y. Tanada of the Division of Invertebrate Pathology, University of California at Berkeley, kindly supplied me with larvae of *Heliothis zea* infected by a microsporidian that he suspected to be *Nosema heliothidis*. These insects were used for further experiments with artificial infections of larvae.

Healthy larvae of *H. zea* were infected by various methods: feeding with contaminated food, microfeeding or microinjection using Dutky's micro-injector. Infected larvae were kept in paper boxes, and the course of infection was examined.

Some larvae were dissected on the 5th and 10th day after infection to check the sequence of tissues infected. Other larvae were kept until death occurred. Both groups of larvae were used to prepare smears and microtome sections. The life cycle of the parasite was studied on smeared preparations

[1] These studies were undertaken in 1958 when the author worked at the University of California as a fellow of the Rockefeller Foundation and were completed at the Institute of Plant Protection in Poland.

fixed in methyl alcohol and stained with 1% Giemsa's solution. The larvae used for microtome sectioning were fixed in Bouin's fluid, embedded in paraffin, and then 5 μ thin sections were prepared and stained according to Delafield's hematoxylin and counterestained with eosin.

Results and interpretation

Parasite identity

There are slight differences between Kramer's 1959 observations and my own. The size of spores given by Kramer is somewhat larger than observed in my studies (Table 1). Fixed with osmium tetroxide and stained with Giemsa's solution the spores in Kramer's material were 2.5 to 5.5 μ long (typical 4.5 to 5.5) and 2.0 to 3.5 μ (typical 3.0 to 3.5) wide. Unfortunately, Kramer did not give the size of fresh spores.

Table 1

Frequency distribution of the length of spores of *Nosema heliothidis* L. et S.

Origin of spores	Dimensionable groups (in microns)						
	2.6—3.0	3.1—3.5	3.6—4.0	4.1—4.5	4.6—5.0	5.1—5.5	5.6—6.0
Kramer's slide with stained spores	11	15	14	8	1	1	
Spores in my material:							
fixed and stained	1	12	23	11	3		
fresh spores		5	12	18	9	4	2

In my studies I have found that spores fixed with absolute methyl alcohol and then stained with Giemsa's solution are 2.8 to 5.0 μ long and 2.0 to 3.0 μ wide. The fresh spores measured in water are 3.3 to 6.0 μ long and 2.5 to 3.5 μ wide (Table 1).

There were also some differences in the staining of spores in Kramer's studies and during my work. Kramer observed that spores fixed with osmium tetroxide were stained deeply and uniformly with Giemsa's solution, while in my material only the central part (the sporoplasm) was stained deeply while the spore wall was unstained (Pl. I 1).

I assume that these differences are due to a different technique used in the fixation of spores. Kramer had used a solution of osmium tetroxide, while in my research methyl alcohol was applied.

In general, measurements done by Kramer and myself are in accordance with the figures given by Lutz und Splendore 1903, 1904. In their original description spores of *N. heliothidis* were 2.5 to 5.5 μ long and 1.7 to 2.0 μ wide. It is rather difficult to judge whether these data refer to fresh or fixed spores.

Another problem requiring careful attention is the tissue attacked by *Nosema heliothidis* in its host. Lutz und Splendore 1904 gave no information on the tissue infected by the parasite. Pinto 1925 was the

first to report that *N. heliothidis* infects the intestine of the host. Kramer 1959 reported that *N. heliothidis* infected only the ventriculus of *Heliothis zea* and *H. virescens*. However, this author expressed the following opinion: "Whether or not the parasite is restricted to this site is not certain. It seems possible that other organs; e.g., Malpighian tubes, may be involved in advanced infections although this was not observed."

In my studies I have found that almost all tissues may be subjected to an attack of the parasite. When spores were transovarially inherited the infection was always heavy and various tissues were involved in infection. Also in artificial infections I observed heavier infections of larvae than Kramer had done. I assume this was due to different methods of artificial infections. I applied the microfeeding technique and therefore a great number of spores (up to 697 000) was distributed to each larvae, while Kramer fed larvae with contaminated food. In experimental infection performed during my studies I observed that when larvae were infected with large number of spores the infection developed much quicker than in larvae infected with small number of spores. It may also be pointed out that when spores are inherited congenitally the infection is always very heavy and almost all tissues of the host are attacked.

Basing on the facts discussed above I consider that the microsporidian studied by me is *Nosema heliothidis* Lutz et Splendore, the same as studied by Kramer 1959.

Histopathology of infection

There is no way of making a certain diagnosis of the microsporidian infection of larvae of *H. zea*. However, it may be pointed out that diseased larvae are not as active as healthy ones and they loose appetite. The critical period in the life cycle of infected larvae is pupation and a high percentage of diseased larvae fail to pupate, or abnormal pupae are produced.

In order to make a sure diagnosis it is necessary to examine larvae microscopically as several tissues reveal specific changes when they are attacked by the parasite. This is especially clear when examining the salivary gland and the midgut. Normal tissues are translucent while infected organs are opaque or whitish in color. Under low power of the microscope infected cells of the salivary glands are looking dark (Pl. I 2).

The dark color of the organs is due to the great number of spores of the parasite, that fill the cells. In a very heavy infection most of the cells are broken and spores are released into the lumen of the salivary glands.

When smeared and microtome preparations are examined spores and other developmental stages of *Nosema heliothidis* may be frequently observed in hemocytes (Pl. II 3, 4, 5). Infected hemocytes show hypertrophy as they are much larger than healthy ones. At the advanced stage of infection attacked hemocytes become degenerated and destroyed (Pl. II 5).

Muscles are rather mildly infected and the infection is rather focal (Pl. II 6, III 8). In some cases the muscle is free but the parasite intensively multiples in the epithelial sheat of the muscle (Pl. III 10). On the other hand, the fat body is heavily infected (Pl. II 7).

The midgut epithelium is especially heavy infected, and in a very advanced infection almost all epithelial cells are destroyed (Pl. III 9). This is due to the fact that quite often simultaneous microsporidian and virus infections occur.

In Pl. IV 11 one can see spores of *Nosema heliothidis* and polyhedra of the cytoplasmic polyhedrosis virus (*Smithiavirus* sp.) in epithelial cells of the midgut of *Heliothis zea*. The detailed studies of simultaneous microsporidian and virus infections are published elsewhere (L i p a 1968).

Quite often a heavy infection is observed in the tracheal matrix where spores and polyhedra of the nuclear polyhedrosis virus (*Borrelinavirus* sp.) occur together (Pl. IV 12).

The adults, both male and female were observed to be infected by the parasite. The main site of infection in adults was the fat body, but the parasite was also observed in ovaries and testes.

Effect on the host and host specificity

Several tests on artificial infections of various insects were performed using spores of *Nosema heliothidis*. However, positive infections were received only among larvae of *Heliothis zea;* other insects were not susceptible to infection (Table 2).

Table 2

Infectivity of spores of *Nosema heliothidis* L. et S. to various insects

Test insect	Method of infection	Number of tested insects	Number of infected insects
Bombyx mori	CF	10	0
Galleria mellonella	MI	25	0
Junonia coenia	MI	5	0
Heliothis zea	MF	20	20
Heliothis zea	MI	20	20
Heliothis zea	CF	14	11
Peridroma margaritosa	MF	11	0
Pseudalitia unipunctata	MF	17	0
Tenebrio molitor	MI	10	0
Tenebrio molitor	MF	10	0

Abbreviations: CF — contaminated food; MF — microfeeding; MI — microinjection.

The longevity of infected larvae of *Heliothis zea* is highly suppressed by the parasite. On an average the death occurred on the 12th or 14th day after infection, but it varied from 8 to 22 days depending on the number of spores introduced to the larvae. In our tests we applied from 14 133 to 697 000 spores per larvae.

When a fifth instar larva is infected with a small number of spores it often happens that the larva is able to pupate and develop into an adult. The infection, however, progresses and gonads frequently become infected. In fact, the transovarial transmission is a very important way in the epizootiology of microsporidian infection caused by *Nosema heliothidis*. Due to this fact freshly hatched first instar larvae of *Heliothis zea* are already infected.

Nomenclature problems

There are some questions about the specific name of *Nosema heliothidis*

and its hosts that should be explained. Lutz und Splendore 1904 evidently used two names for the same microsporidian that is *Nosema armigerae* and *Nosema heliothidis*, both from *Heliothis armigera* Hübner. Pinto 1925 listed both species in his paper on protozoan parasites of insects in Brazil. When the paper of Lutz und Splendore 1904 is carefully examined it is evident that both species must be synonymized. Although the name *Nosema armigerae* was used earlier (basing on the number of pages) the name *Nosema heliothidis* has been in common use for several years. Therefore the name *Nosema heliothidis* should be accepted and *Nosema armigerae* should be considered as a synonym of the former.

Weiser 1946, 1961 suggested that *Nosema heliothidis* should be identified with *Nosema eubules* Lutz et Splendore, 1903 described from *Catopsilia eubule*. He based his assumption on the similarity of spore size of both microsporidians as Lutz und Splendore reported that spores of *N. heliothidis* were 2.5—5.5×2.0—3.5 μ, while spores of *N. eubules* were 2—5×1.0—2.5 μ.

It is rather difficult to clear up this problem completely as slides with spores of *Nosema eubules* used by Lutz und Splendore are not available. In my opinion, however, these two microsporidians are separate and independent species although their morphological features are somewhat similar. It should be remembered that both host insects are systematically not related; the *Heliothis* spp. belong to the family *Noctuidae* while the *Catopsilia eubule* belongs to the family *Pieridae*. Therefore there is no justification to identify *Nosema heliothidis* with *Nosema eubules* and these two microsporidians should be considered as separate species. However, I fully agree with Dr. Weiser that it would be highly desirable to make a detailed study of microsporidians described by Lutz und Splendore from *Lepidoptera*. Descriptions of many species presented by Lutz und Splendore include only the size of spores and name of host insect but in the present view on the taxonomy of *Microsporidia* these characters are frequently insufficient to differentiate a new species.

Although it is generally believed that the original finding of *Nosema heliothidis* was made in *Heliothis armigera* due to studies by Common 1953 and Todd 1955, this view should be changed. While revising the *Heliothis* genus, Todd 1955, pointed out that *Heliothis armigera* (Hübner) was distributed in Palearctic, that is in Europe, Africa, Asia and the Far East but it does not occur in South America where the original finding of *Nosema heliothidis* was made by Lutz und Splendore 1903, 1904. In Brazil *Heliothis zea* (Boddie) and *H. gelotopoeon* (Dyar.) occur while in North America — *Heliothis virescens* (Fabricius) and *H. zea* (Boddie) are met. Consequently, it must be stated that *Heliothis armigera* was not, so far, found as the host for *Nosema heliothidis* and that this microsporidian was originally described in *Heliothis gelotopoeon* (Dyar.) or/and *H. zea* (Boddie).

So far, *Nosema heliothidis* was reported from Brasil (Lutz und Splendore 1903, 1904; Pinto 1925), and United States (Kramer 1959; Lipa and Steinhaus 1960).

Summary

Several new data on the development and pathogenicity of *Nosema heliothidis* Lutz et Splendore parasitizing in *Heliothis zea* (Boddie) are given. Fresh spores of this microsporidian are 3.3—6.0×2.5—3.5 μ; fixed and

stained spores are 2.8—5.0×2.0—3.0 μ. The parasite causes a general infection and attacks the midgut, hemocytes, salivary glands, muscles, gonads and other tissues. *N. heliothidis* is a lethal parasite of *H. zea* and artificial infections were easily performed by administering spores per os. Transovarial transmission of this parasite is very common. Synonyms, host insects and distribution of *N. heliothidis* have been discussed.

STRESZCZENIE

Uzyskano szereg nowych danych o rozwoju i chorobotwórczości pierwotniaka *Nosema heliothidis* Lutz et Splendore pasożytującego w *Heliothis zea* (Boddie) w USA. Świeże spory mierzą 3.3—6.0×2.5—3.5 μ; utrwalone i barwione spory mierzą 2.8—5.0×2.0—3.0 μ. Pasożyt wywołuje ogólną chorobę i zaraża jelito, krwinki, gruczoły ślinowe, mięśnie, gonady i inne tkanki. *N. heliothidis* jest letalnym pasożytem *H. zea* i jest wysoce zaraźliwa dla owadów przy zarażaniu per os. Transowaryjne przenoszenie się pasożyta ma bardzo często miejsce. Omówiono synonimy, owady żywicielskie i rozprzestrzenienie *N. heliothidis*.

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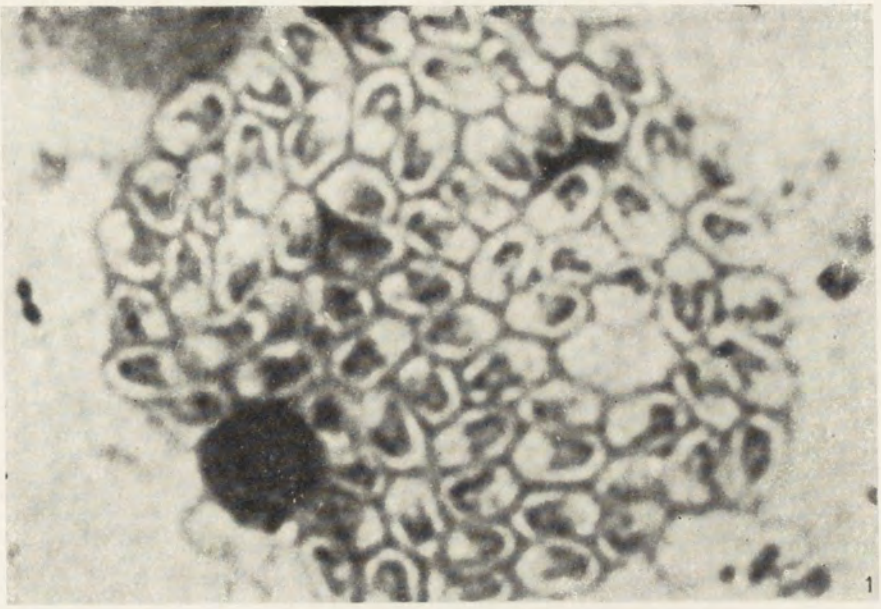
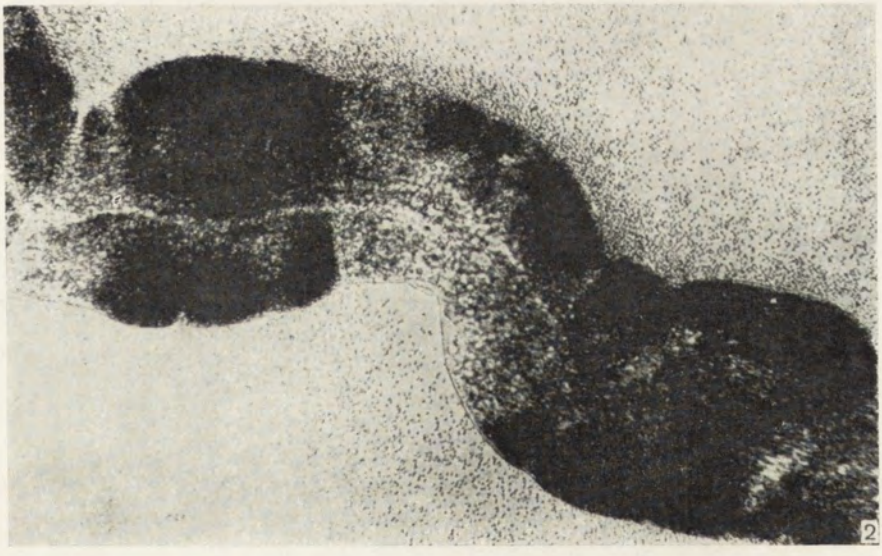
EXPLANATION OF PLATES I—IV

Nosema heliothidis L. et S. developing in *Heliothis zea* (Boddie)

1: A group of spores
2: Salivary glands heavily infected
3: Schizonts inside the hemocytes
4: Free schizonts
5: Hemocyte destroyed by spores
6: A group of spores in the muscle
7: Spores seen as white spots in fat body
8: Two groups of spores in muscles and nuclei of fat body infected with nuclear polyhedrosis virus (*Borrelinavirus* sp.)
9: Midgut epithelium completely destroyed by microsporidian
10: Spores in the epithelial envelope of muscle
11: Spores in the epithelial cells of midgut together with inclusion bodies of the cytoplasmic polyhedrosis virus (*Smithiavirus* sp.)
12: Spores seen as white spots and inclusions of nuclear polyhedrosis virus seen as dark spots in the tracheal matrix.





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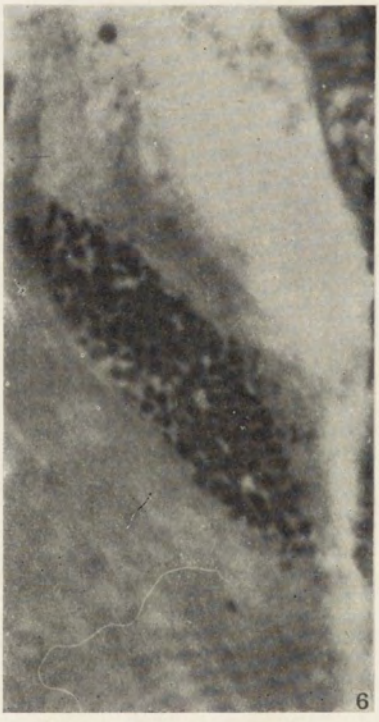

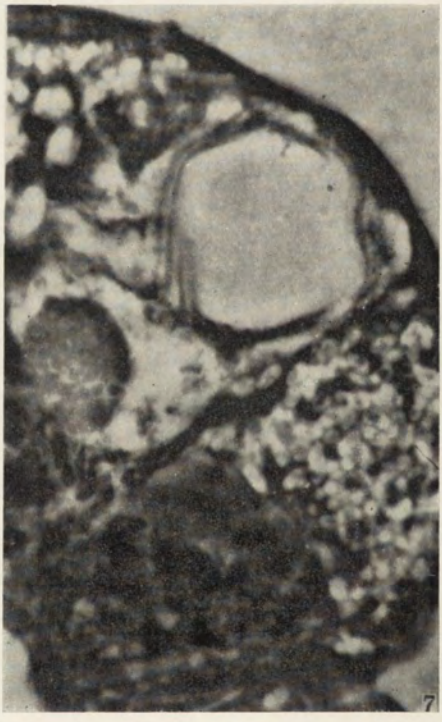

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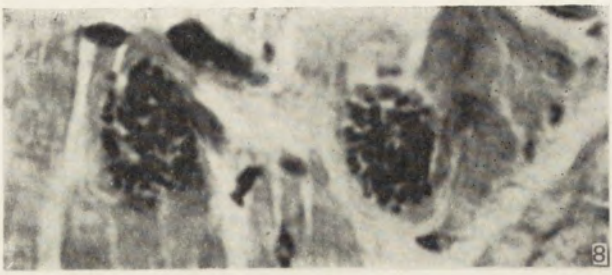

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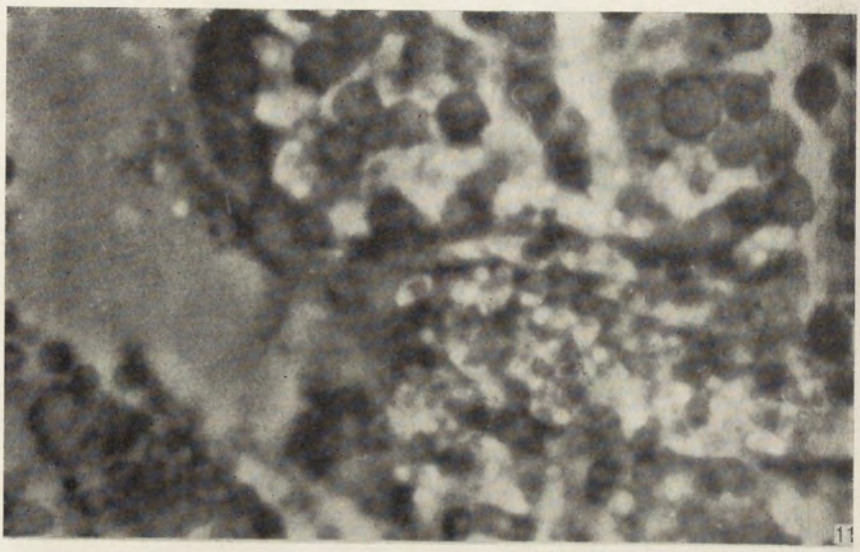

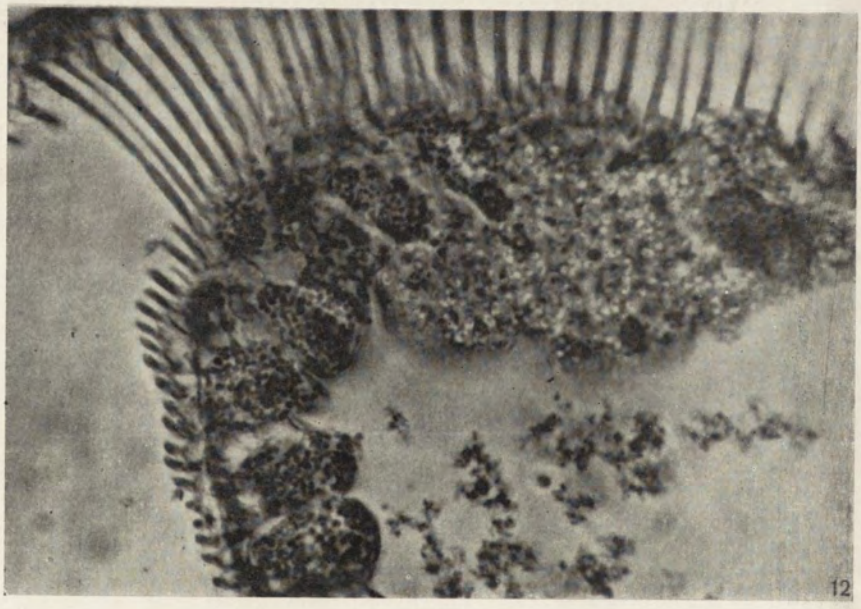

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Report on identification of *Protozoa* pathogenic for insects in the Soviet Union (1961—1966), with descriptions of some new species

Sprawozdanie z identyfikacji pierwotniaczych chorób owadów w Związku Radzieckim (1961—1966) i opisy nowych gatunków

For several years the All-Union Institute of Plant Protection in Leningrad was receiving diseased insects for diagnosis. In case of protozoan pathogens the parasites were identified to genus, or probable species, and stored for future study. The results of some of the preliminary identifications were published by Evlakhova i Shvetsova 1965.

In 1965 and 1966, while junior author worked at the All-Union Institute of Plant Protection in Leningrad as a visiting scientist, we made a further study as to the identity of the stored protozoans. The results of this work are included in this paper. Besides, the authors included the results of examination of insects collected by themselves or obtained from other sources in 1965—1966.

Methods

The handling of the material depended on its type and origin. In case of dead insects only smeared preparations could be performed and data on tissues attacked were rarely available. On the other hand, living insects were dissected, and their gut and other tissues were used to prepare microscopic slides.

Spores and developmental stages of microsporidians were studied on smeared preparations fixed in methyl alcohol and stained with 1% Giemsa's solution. Gregarines were observed and measured in fresh preparations.

Results

Protozoan pathogens of thirteen insect species were studied in detail (Table 1). They included only two gregarine species while others were infected with microsporidians. Almost half of them represented new records and three recorded microsporidians were described as new species.

Table 1

A list of studied protozoans and their insect hosts

Insect	Protozoan
Microsporidian infection	
Antherea pernyi Guer.	*Nosema bombycis* Nägeli and *Nosema* sp.
Argyresthia conjugella Zell.	*Thelohania argyresthiae* sp. n.
Argyresthia pygmaella Hb.	Unidentified microsporidian
Carpocapsa pyrivora Danil.	*Nosema carpocapsae* Paillot
Chorthippus albomarginatus DeGeer	*Nosema locustae* Canning
Dasychira pudibunda L.	*Thelohania dasychirae* sp. n.
Euproctis chrysorrhoea L.	*Nosema lymantriae* Weiser
Hylemia antiqua Meig.	*Octosporea* sp.
Laspeyresia strobilella L.	*Nosema* sp.
Lymantria dispar L.	*Nosema lymantriae* Weiser
Phalera bucephala L.	*Nosema phalerae* sp. n.
Pissodes piceae Ill.	*Nosema* sp.
Thaumetopoea processionea L.	*Plistophora* sp.
Tortrix viridana L.	*Nosema tortricis* Weiser
Gregarine infections	
Gryllus domesticus L.	*Leidyana gryllorum* Watson
Tenebrio molitor L.	*Gregarina cuneata* Stein

Microsporidian infections

Antherea pernyi Guer.

Microsporidian: *Nosema bombycis* Nageli and unidentified species.
Locality record: Leningrad, 1958—1959.

A laboratory culture of *A. pernyi* kept in the Laboratory of Microbiological Methods at the All-Union Institute of Plant Protection was found to be heavily infected in 1958—59 with two microsporidians (Evlakhova i Shvetsova 1965). One of them was identified as *Nosema bombycis* Nageli, 1857, while the other differed from *N. bombycis* and *Plistophora balbiani* Veber, 1963 known from this insect. All moths developing from larvae were infected by parasites.

Argyresthia conjugella Zell.

Microsporidian: *Thelohania argyresthiae* sp. n.
Locality record: Vilnius Region, 26.VII.1966.

Dead larvae were collected by M. M. Gerasimovič on July 26, 1966 in the region of Vilnius and submitted in their web nests. At the microscopic examination it was found that a few larvae were infected with a new microsporidian.

The type of sporogony and pansporoblasts indicated that this species belonged to the genus *Thelohania* as eight spores are produced from each

sporont. Fixed and stained spores are from 3.0 to 6.0 (mainly 3.1—4.0) μ long and 2.0—3.1 μ wide (Table 2) (Pl. I 1). In fresh preparations they appeared in groups of eight, but only some of them preserved this form after staining. This parasite was observed in the fat body of dead larvae.

Table 2

Frequency distribution of the lenght of one sample of 50 spores of *Thelohania argyresthiae* sp. n.

Classes (microns)	2.6—3.0	3.1—3.5	3.6—4.0	4.1—4.5	4.6—5.0	5.1—5.5	5.6—6.0
Frequency	3	19	23	1	1	2	1

This is the first record of microsporidian infection in *Argyresthia conjugella* and apparently a new species is involved. Therefore we propose the name *Thelohania argyresthiae* sp. n. for it.

Argyresthia pygmaella Hb.

Microsporidian: Unidentified species.
Locality record: Krasnodar Country, 1966.

In autumn of 1966 Dr A. Evlakhova submitted dead larvae of *A. pygmaella* she had collected in Krasnodar in July, 1966. The larvae were dry and their condition did not allow to make a taxonomic study on the microsporidian involved.

Spores of the microsporidian from *A. pygmaella* are almost twice smaller than of *Thelohania argyresthiae* sp. n. Fixed and stained spores are from 1.6 to 2.3 μ long and 1.1—1.3 μ wide (Pl. I 2). The condition of material available for our study did not allow to determine the generic position of the involved microsporidian.

Carpocapsa pyrivora Danil

Microsporidian: *Nosema carpocapsae* Paillot.
Locality record: Krasnodar Country 1965.

The size and shape of spores of the microsporidian involved (Pl. I 3) indicate that this is *Nosema carpocapsae* Paillot, 1939, a well known parasite of *Carpocapsa pomonella* L. This is the first record of microsporidian infection of *Carpocapsa pyrivora*.

Chorthippus albomarginatus DeGeer

Microsporidian: *Nosema locustae* Canning.
Locality record: Irkutsk Region (Siberia), 1965.

Dead insects were supplied by Dr G. A. Popov of the same Institute. At the microscopic examination large oval spores were observed which were well stained with Giemsa's solution (Pl. I 4, 5). The size of spores varied from

3.5 to 5.0 μ in length and 2.0 to 3.0 μ in width. This is the first record of microsporidian infection of *Chorthippus albomarginatus* and the parasite is identified as *Nosema locustae* Canning, 1953, known from grasshoppers.

Dasychira pudibunda L.

Microsporidian: *Thelohania dasychirae* sp. n.
Locality record: Suchumi (Caucasus), 1960.

Insects were collected as second instar larvae in Caucasus, then brought to the Biological Institute at Peterhoff, close to Leningrad, and used for experimental purposes. Out of sixty dead and examined larvae two were found to be infected with a new species of a microsporidian: one larva was heavily and the other weakly infected. In fresh water preparations of the fat body of dead larvae pansporoblasts, typical of *Thelohania* genus, were observed.

Schizogony and sporogony is typical of the genus *Thelohania* as eight spores are produced from each sporont. Spores are oval 3.2 to 5.8 μ long and 2.0 to 3.0 μ wide (Table 3) (Pl. II 6). Some of them have an irregular shape which is rather characteristic in the genus *Thelohania*.

Table 3

Frequency distribution of the length of one sample of 50 spores of *Thelohania dasychirae* sp. n.

Classes (microns)	3.1—3.5	3.6—4.0	4.1—4.5	4.6—5.0	5.1—5.5	5.6—6.0
Frequency	6	39	2	5	—	1

Evlakhova i Shvetsova 1965 first reported *Thelohania* infection in *D. pudibunda* and we used the same material. As this microsporidian does not resemble any other protozoans found in related insects we consider it as a new species and propose the name *Thelohania dasychirae* sp. n. for it.

Euproctis chrysorrhoea L.

Microsporidian: *Nosema lymantriae* Weiser.
Locality record: Stanislav (Carpathian Region), 1961.

In the fat body of infected larvae narrow and strongly elongate spores were observed. They are 3.2 to 7.0 μ long and 1.0 to 2.0 μ wide (Pl. II 7). The features of this microsporidian indicate that this is *Nosema lymantriae* Weiser, 1957.

Hylemyia antiqua Meigen

Microsporidian: *Octosporea* sp.
Locality record: Sverdlovsk Region (Ural), 1959.

A few puparia were submitted by Mrs. A. V. Korobejnikova from the Plant Protection Laboratory at Sverdlovsk. The tissues of the dead pupae and adult flies, closed inside puparia, were completely destroyed by a microsporidian. Spores were greatly elongated and measured 3.7×0.6 μ. The type of schizogony as well as the shape and size of spores indicate that this microsporidian belongs to the genus *Octosporea*.

The only known species from this genus parasitizing *Muscidae* is *Octosporea muscaedomesticae* Flu described from *Musca domestica* L. The onion fly *Hylemyia antiqua* has never been reported to be the host for *O. muscaedomesticae*. Therefore, the question, whether *Octosporea* sp. is a new species or identical with *O. muscaedomesticae*, ought to be studied.

The finding of the studied *Octosporea* sp. in *H. antiqua* was previously reported by Evlakhova i Shvetsova 1965.

Laspeyresia strobilella L.

Microsporidian: *Nosema* sp.
Locality record: Leningrad Region and Kirov Region 1966 and 1967.

Infected insects were collected by Dr. G. V. Stadnickij in cones of *Picea* spp. in Leningrad region in 1966 and 1967. Living spores of the investigated microsporidian are oval 2.7—4.2 μ long and 1.2—2.2 μ wide (Pl. II 8). The type of schizogony and sporogony indicates that this microsporidian belongs to the genus *Nosema* and evidently is a new species. However, as data on infected tissues are not available we designate this species only by the generic name *Nosema* sp.

Lymantria dispar L.

Microsporidian: *Nosema lymantriae* Weiser.
Locality record: Sverdlovsk Region, 1965.

Insects were collected and submitted by Mrs. S. P. Berdennikova. The main site of infection were silk glands. The spores were elongated and from 3.1 to 6.1 μ long and 1.0 to 2.0 μ wide (Pl. III 9). Young spores were binucleated while mature spores had one nucleus. Due to its characteristic this microsporidian is identified as *Nosema lymantriae* Weiser, 1957.

The infection of *L. dispar* had an epizootic character and in the following year 1966 in many localities of the surveyed area the mortality of insects was about 80%.

Phalera bucephala L.

Microsporidian: *Nosema phalerae* sp. n.
Locality record: Cernovcy (Karpathian Region), 1961.

Spores observed in the fat body of infected insects were 2.2 to 4.5 μ long and 1.3 to 2.0 μ wide (Pl. III 10). The polar filament was 40 μ long. Evlakhova i Shvetsova 1965 were the first to report an unidentified microsporidian infection in *Phalera bucephala*. Our studies are based on the same material.

The examination of the life cycle of the microsporidian involved indicates that it belongs to the genus *Nosema*. This is the first record of a microsporidian infection in this insect and evidently a new species is involved. Therefore we propose the name *Nosema phalerae* sp. n. for it.

Pissodes piceae Ill.

Microsporidian: *Nosema* sp.
Locality record: Teberda (Caucasus).

Infected larvae and beetles were collected by Mrs. T. M. Gurjanova. Spores of the observed microsporidian are elongate and well stained with Giemsa's solution. They are 3.6 to 5.0 μ long and 2.0 to 2.9 μ wide (Pl. III 11). In young spores two red nuclei are well seen. No data on host tissues attacked are available. Due to its morphological features and lack of sufficient material, this species is designated as *Nosema* sp.

Thaumetopoea processionea L.

Microsporidian: *Plistophora* sp.
Locality record: Carpathian Region, 1961.

Spores are oval and rather small. When stained they are 2.0 to 3.1 μ long and 1.0 to 2.0 μ wide (Table 4) (Pl. III 12). Evlakhova i Shvetsova 1965 previously recorded *Plistophora* infection in this insect, and we used the same material in our work. However, lack of suffcient material has not allowed to make detail taxonomic studies on this species and therefore it is designated only as *Plistophora* sp.

Table 4

Frequency distribution of the length of one sample of 50 spores of *Plistophora* sp. from *Thaumetopoea processionea* L.

Classes (microns)	1.1—1.5	1.6—2.0	2.1—2.8	2.6—3.0	3.1—3.5
Frequency	2	14	18	14	2

Tortrix viridana L.

Microsporidian: *Nosema tortricis* Weiser.
Locality record: Cherson, 1963.

Infected larvae were collected and submitted by Mrs. L. M. Zelinskaja. Spores observed in the fat body of larvae were about 4 μ long and 2 μ wide (Pl. III 13). Due to its features this microsporidian is identified as *Nosema tortricis* Weiser, 1956.

Gregarine infections

Paralely to studies of microsporidians some limited investigations were carried on gregarine infections of insects. The results of these studies have been partially published (Lipa 1966, 1968), while others are included in this paper.

Insects examined during these studies were collected by the authors or kindly supplied by the staff members of the All-Union Institute of Plant Protection in Leningrad, Severtzov's Institute of Animal Morphology in Moscow, and Institute of Plant Protection in Kiev.

The following insect species were examined, and in parentheses the number of specimens is given: *Coleoptera* — *Anisoplia austriaca* Hrbst. (9), *Rhyzopertha dominica* F. (8), *Prionus coriarius* L. (7), *Trichius fasciatus* L. (9), *Potasia metallica* Hrbst. (11), *Tenebrio molitor* L. (20); *Diptera* — *Tipula scripta* Meig. (11); *Orthoptera* — *Blatella germanica* L. (5), *Gryllus domesticus* L. (19).

Gryllus domesticus L.

Gregarine: *Leidyana gryllorum* Watson.
Locality record: Moscow, laboratory culture, 3.II.1966.

The examined insects were obtained from a laboratory culture of the Severtzov's Institute of Animal Morphology in Moscow. Out of 19 examined insects, 9 were found to be infected with the gregarine identified as *Leidyana gryllorum* Watson, 1916.

Gamonts solitary and elongate. Maximum length of observed gamonts 255 μ; maximum width 114 μ. Ratio LP:TL = 1:4; ratio WP:WD = 1:1.2—1.7 (Table 5).

Table 5

Measurements of gamonts of *Leidyana gryllorum* Watson (in microns)

LP	LD	WP	WD	TL	LP:TL	WP:WD
36	149	48	70	185	1:5	1:1.4
18	158	53	84	176	1:9	1:1.6
44	211	70	114	255	1:6	1:1.4
35	194	62	106	229	1:6	1:1.7
26	114	44	53	140	1:5	1:1.2
31	141	40	70	172	1:5	1:1.7
53	158	48	62	211	1:4	1:1.2

Abbreviations:
LP — length of protomerite
LD — length of deutomerite
WP — width of protomerite
WD — width of deutomerite
TL — total length of gamont
LP:TL — ratio length of deutomerite to total length
WP:WD — ratio width of protomerite to width of deutomerite

The epimerites are irregular, translucent and clearly seen in the majority of trophozoites. The protomerites are oval with a thin epicyte. The ectoplasm is dense, granular and dark-brown. Septum and constriction clearly seen.

Deutomerite widest in half-length and narrowing toward the end. Endoplasm dense and black. The nucleus about 30 μ in diameter is located in the half length of the body.

The cysts without sporeducts are 200 μ in diameter.

Tenebrio molitor L.

Gregarina: *Gregarina cuneata* Stein.
Locality record: Kiev, 2.III.1966.

Insects were received from a laboratory culture from Zabolotny's Institute of Microbiology and Virology in Kiev. Out of 20 larvae 2 were found to be infected with a gregarine that was identified as *Gregarina cuneata* Stein.

The gamonts were biassociative. Maximum length of observed gamonts 386 μ; maximum width 159 μ. Ratio LD:TL = 1:4; ratio WP:WD = 1:1.6—1.9 (Table 6).

Table 6

Measurements of gamonts of *Gregarina cuneata* Stein (in microns)

LP	LD	WP	WD	TL	LP:TL	WP:WD
85	255	95	140	340	1:4	1:1.6
88	298	88	159	386	1:4	1:1.9

Protomerite elongate with a narrow region in the center. Endoplasm dark-brown. Deutomerite cylindrical with round end. Ectoplasm thin and clear but endoplasm dark. Nucleus not seen. The cysts were up to 234 μ in diameter.

Protozoans not identified as to genus

We had at our disposal also materials or notes kept in various laboratories of the All-Union Institute of Plant Protection on protozoan infections of insects. Unfortunately, it was not possible to examine these materials in a more detailed way. At any rate we have decided to include these data in our paper as it may attract attention of investigators to collect and examine such insects, whenever available. Some of the insects listed below were already reported as hosts for protozoans by Evlakhova i Shvetsova 1965.

Unidentified microsporidians were recorded in:

Anisoplia austriaca Herbst.
Cheimatobia brumata L.
Dioryctria splendidella H.-S.
Notodonta trepida Esp.
Panolis flammea Schiff.

Unidentified gregarines were recorded in:
Amphimallon solstitialis L.
Ophonus pubescens Müll.
An unidentified protozoan was recorded in *Eurygaster integriceps* Put.

Summary

The authors reported the results of identification of protozoans pathogenic for insects in the Soviet Union during 1961—1966. Materials used in the course of this study were stored in the All-Union Institute of Plant Protection in Leningrad or collected by the authors. A total number of 23 insect species were examined during this work. Microsporidian infections were recorded in: *Anisoplia austriaca* Herbst., *Antherea pernyi* Guer., *Argyresthia conjugella* Zell., *A. pygmaella* Hb., *Carpocapsa pyrivora* Danil., *Cheimatobia brumata* L., *Chorthippus albomarginatus* DeGeer, *Dasychira pudibunda* L., *Dioryctria splendidella* H.-S., *Euproctis chrysorrhoea* L., *Hylemyia antiqua* Meig., *Laspeyresia strobilella* L., *Lymantria dispar* L., *Notodonta trepida* Esp., *Phalera bucephala* L., *Pissodes piceae* Ill., *Thaumetopoea processionea* L., *Tortrix viridana* L., *Panolis flammea* Schiff. Gregarine infections were observed in: *Amphimallon solstitialis* L., *Gryllus domesticus* L., *Ophonus pubescens* Müll., and *Tenebrio molitor* L. An unidentified protozoan was observed in *Eurygaster integriceps* Put. Most insects have been reported as hosts of protozoans for the first time. Three new microsporidians have been described: *Thelohania argyresthiae* sp. n. from *Argyresthia conjugella* Zell., *Thelohania dasychirae* sp. n. from *Dasychira pudibunda* L., and *Nosema phalerae* sp. n. from *Phalera bucephala* L.

STRESZCZENIE

Autorzy zestawiają wyniki identyfikacji pierwotniaków pasożytujących w owadach w Związku Radzieckim za lata 1961—1966. Materiały te były przechowywane we Wszechzwiązkowym Instytucie Ochrony Roślin w Leningradzie lub zostały zebrane przez autorów. Ogólnie przebadano 23 gatunki owadów. Mikrosporidia zanotowano w owadach: *Anisoplia austriaca* Herbst., *Antherea pernyi* Guer., *Argyresthia conjugella* Zell., *A. pygmaella* Hb., *Carpocapsa pyrivora* Danil., *Cheimatobia brumata* L., *Chorthippus albomarginatus* DeGeer, *Dasychira pudibunda* L., *Dioryctria splendidella* H.-S., *Euproctis chrysorrhoea* L., *Hylemyia antiqua* Meig., *Laspeyresia strobilella* L., *Lymantria dispar* L., *Notodonta trepida* Esp., *Phalera bucephala* L., *Pissodes piceae* IU., *Thaumetopoea processionea* L., *Tortrix viridana* L., *Panolis flammea* Schiff., Gregaryny zanotowano w: *Amphimallon solstitialis* L., *Gryllus domesticus* L., *Ophonus pubescens* Müll., *Tenebrio molitor* L. Niezidentyfikowanego pierwotniaka stwierdzono w *Eurygaster integriceps* Put. Większość gatunków owadów jest po raz pierwszy notowana jako żywiciele pierwotniaków. Opisano trzy nowe gatunki mikrosporidiów: *Thelohania argyresthiae* sp. n. z *Argyresthia conjugella* Zell., *Thelohania dasychirae* sp. n. z *Dasychira pudibunda* L. i *Nosema phalerae* sp. n. z *Phalera bucephala* L.

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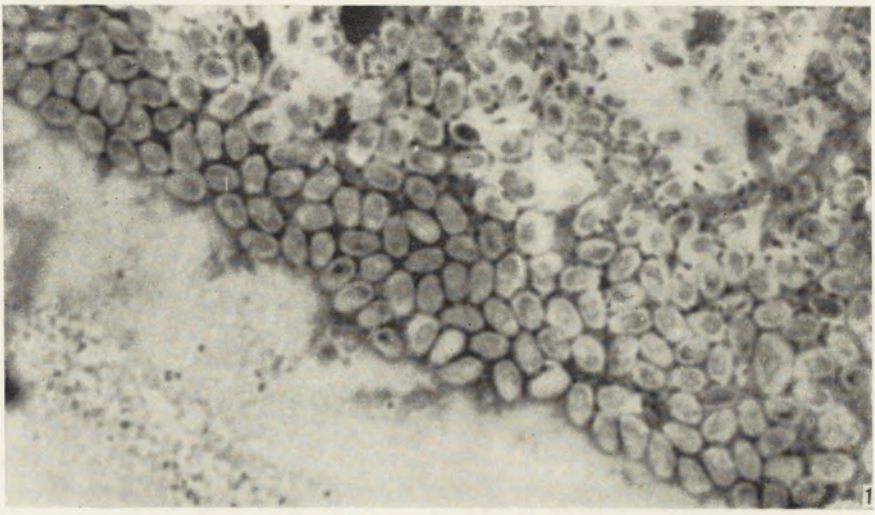
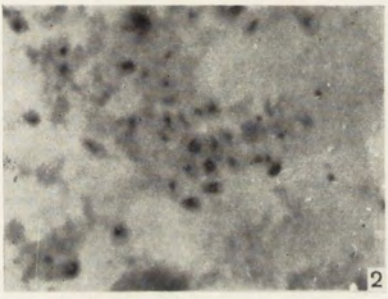
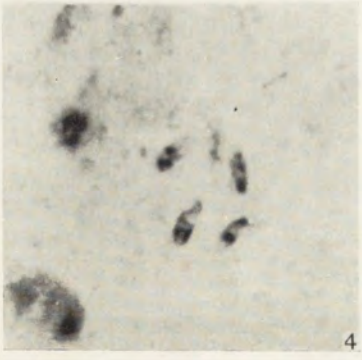
EXPLANATION OF PLATES I—III

1: Spores of *Thelohania argyresthiae* sp. n. from *Argyresthia conjugella,* 1200×
2: Spores of an unidentified microsporidian from *Argyresthia pygmaella,* 1200 ×
3: Spores of *Nosema carpocapsae* Paillot from *Carpocapsa pyrivora,* 1000×
4; 5: Spores (5) and sporonts (4) of *Nosema locustae* Canning from *Chorthippus albomarginatus*
6: Spores of *Thelohania dasychirae* sp. n. from *Dasychira pudibunda,* 1200×
7: Spores of *Nosema lymantriae* Weiser from *Euproctis chrysorrhoea,* 1200×
8: Spores of *Nosema* sp. from *Laspeyresia strobilella,* 1000×
9: Spores of *Nosema lymantriae* Weiser from *Lymantria dispar,* 1200×
10: Spores of *Nosema phalerae* sp. n. from *Phalera bucephala,* 1200×
11: Spores of *Nosema* sp. from *Pissodes piceae,* 1200×
12: Spores of *Plistophora* sp. from *Thaumetopoea processionea,* 1200×
13: Spores of *Nosema tortricis* Weiser from *Tortrix viridana,* 1200×







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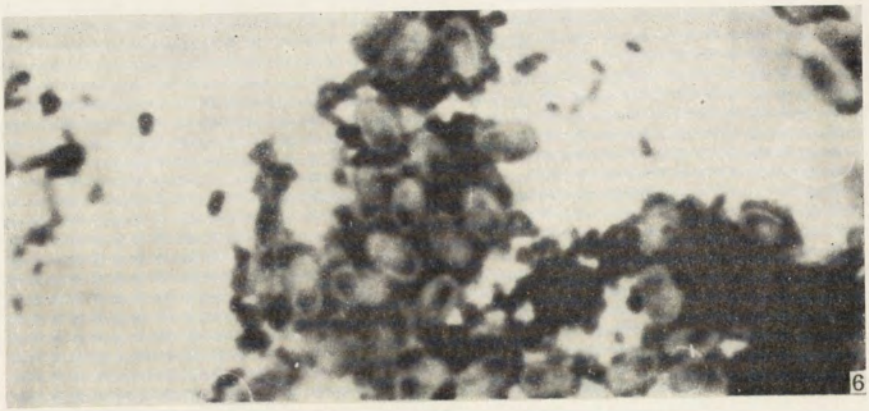

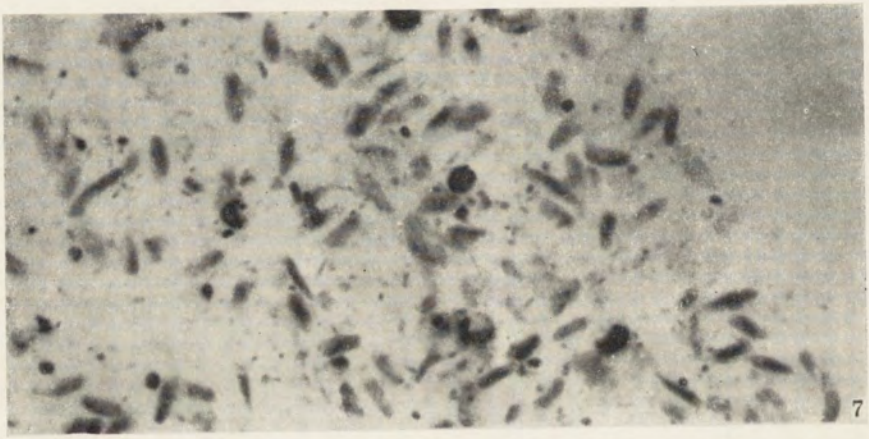

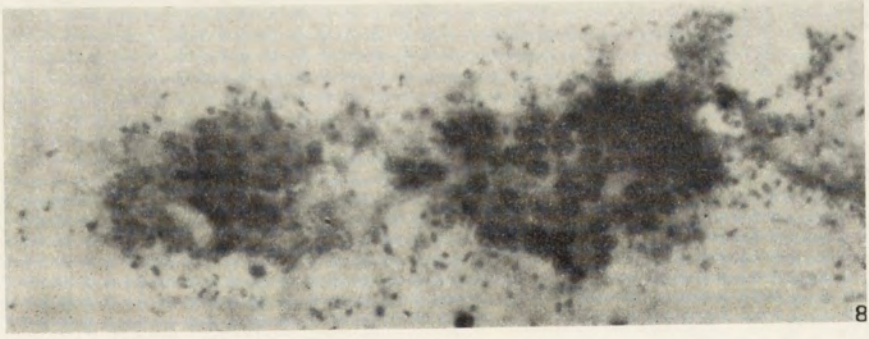

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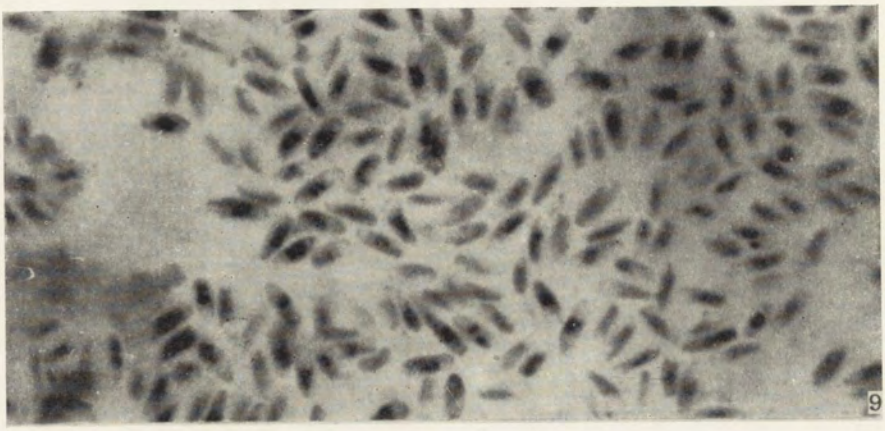

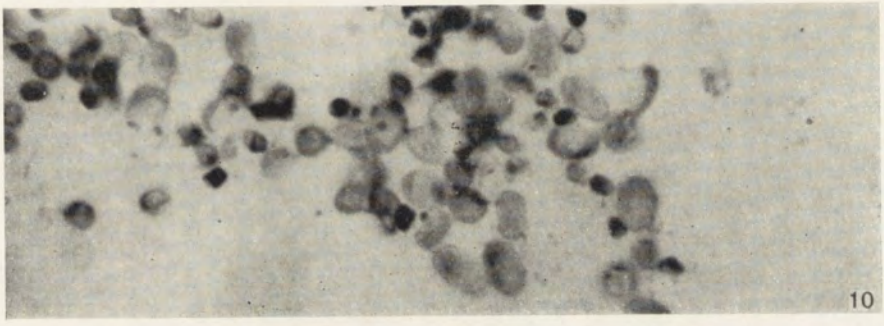

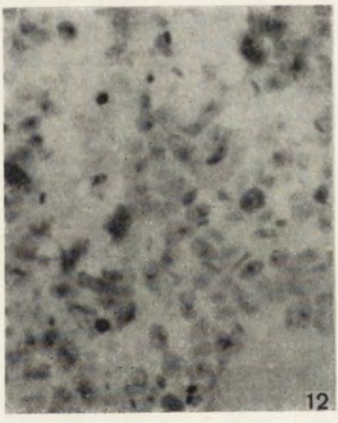

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Digestive enzymes of *Blepharisma intermedium* Bhandary (*Ciliata: Spirotricha*)

Verdauungsfermente von *Blepharisma intermedium* Bhandary *(Ciliata: Spirotricha)*

The distribution and characteristics of different digestive enzymes form an important aspect of physiology of digestion in protozoa. An understanding of the enzyme systems of ciliates is basic to the problem of ciliate nutrition and growth in axenic media.

Although the enzyme systems of *Tetrahymena, Paramecium* (Seaman 1955), *Frontonia* (Vimala Devi 1965) and a few rumen ciliates e.g. *Dasytricha, Isotricha, Epidinium* etc. (see Holz 1964) have been studied, the information available regarding other ciliates is very scanty. In this paper the digestive enzymes of *Blepharisma intermedium* have been described.

Material and methods

The culture of *B. intermedium* was maintained at 25—27°C in hay infusion fortified with Horlicks malted milk. For the study of digestive enzymes, animals were collected and starved for 24 hrs. in Chalkley's medium. The animals were washed in distilled water repeatedly to remove all bacteria and then homogenized. Cell free supernatant was used for the study of enzymes. For incubation, equal volumes of suitable citrate phosphate buffer and an appropriate substrate solution were added to the supernatant. Two types of controls were maintained. In one, the homogenate was omitted or rendered inactive by heating in boiling water for 30 minutes and in the other the substrate was omitted from the incubation medium. The incubation of the mixture was done at 37°C for varying periods of time after adding a drop of toluene. Each incubation mixture was then chemically analyzed for the presence of the substrate and the hydrolytic products of enzyme action. The details of the methods used for the detection of different enzymes are described below.

Carbohydrases

Amylase: The procedure used for the study of amylase activity at different pH values ranging from 4.0—8.0 was that of Krishna 1958. 0.25% starch solution was used as substrate. 0.1 ml of 0.1% NaCl solution and a drop of

toluene was added to all the lots and the mixture was incubated for 90 minutes at 37°C. The reaction was then stopped by adding 0.15 ml of N-hydrochloric acid to each incubation mixture. The amount of starch left unhydrolyzed in each mixture was determined photometrically by treating with potassium iodide-iodine reagent (Smith and Roe 1949). On the basis of optical density the concentration of starch left undigested was calculated. Amylase activity at different pH values was then expressed in terms of the percentage of starch hydrolyzed at the corresponding pH.

The products of hydrolysis of starch were analysed by paper chromatography. 0.1 ml of 1% starch solution was used as substrate. Incubation was done for 18 hrs. at 37°C. The entire mixture was concentrated and subjected to paper chromatography using Whatman No. I filter paper and n-butanol--acetic acid-water (4:1:5 v/v) as solvent. The solvent was run for 48 hrs. by descending technique. The chromatograms were air dried and developed with benzidinetrichloracetic acid reagent at 105°C (Bacon and Edelman 1951). Sugars appeared as brown spots. The position of various sugars was established by comparison with that of reference sugars.

To determine whether the degradation of starch was due to action of amylase or phosphorylase or both, mercuric chloride (M/1000) was used as inhibitor of α-amylase.

Glycosidases: 1% maltose, 5% sucrose and 5% melezitose were used as the α-glucoside substrates for the detection of α-glucosidases. The mixture (at pH 5.6) was incubated for 24 hrs. at 37°C and the material was subjected to paper chromatography. Adequate controls were maintained. In one series, the products of hydrolysis were examined by paper chromatography at the interval of 4 hrs. each during incubation.

Cellobiose, raffinose and lactose (5% solutions) were used as the substrates for the detection of β-glucosidase, α-galactosidase and β-galactosidase respectively. Products of hydrolysis were analysed by paper chromatography.

Proteinases

10% solution of gelatin and 1% solution of egg albumin were used as the substrates for the detection of proteolytic activity at eight different pH values, namely, 3.0, 3.5, 4.2, 5.0, 5.8, 6.6, 7.4, 8.2. Incubation was done for 24 hrs. at 37°C. On completion of incubation, a 0.005 ml sample of each mixture was plotted on Whatman No. I filter paper and the strip was developed in bromophenol blue solution as described by Kunkel and Tiselius 1951. The intensity of blue colour developed is proportional to the amount of protein present. In case of egg albumin, incubation was done for 20—40 hrs., the strip was heated to 100°C for denaturation of proteins before developing.

Esterases

Olive oil and ethyl-butyrate emulsions, prepared as described by Baldwin and Bell 1955, were used as the substrates for the detection of lipases. A drop of phenol red and a few drops of 1% sodium carbonate were added till the incubation medium turned pink. Incubation was done for 72 hrs. at 37°C.

Observations

Carbohydrases

α-amylase: Starch was hydrolyzed at all pH values ranging from 4.0--7.5 (Fig. 1). Maximum hydrolysis occurred at pH 6.0 when 79% of the starch was hydrolyzed within 90 minutes as compared to 29% in *Frontonia* (Vimala Devi 1965). Products of hydrolysis of starch included dextrins, maltose and glucose. Mercuric chloride inhibited the activity of α-amylase in *Blepharisma* as known in other cases (Bailey et al. 1951, Porter 1953).

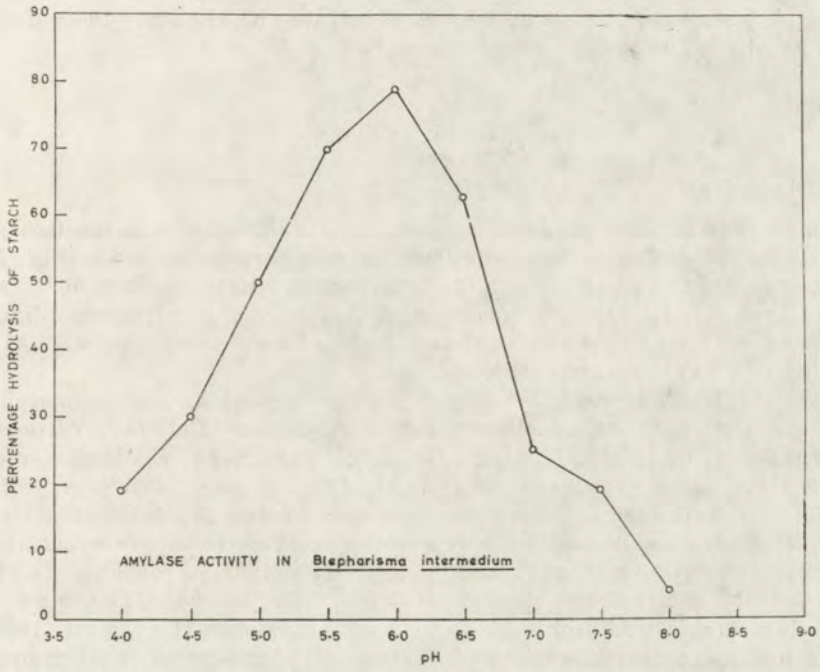

Fig. 1. Graph showing percentage hydrolysis of starch at different pH values

Glycosidases: The hydrolysis of α-glucoside substrates, maltose, sucrose and melezitose by the homogenate of *Blepharisma* yielded glucose, glucose and fructose, and glucose and turanose respectively. Thus the invertase of *Blepharisma* is of α-glucoside type as described by Neuberg and Mandl 1950.

When the products of hydrolysis of sucrose were subjected to paper chromatography at intervals of 4 hrs., another oligosaccharide sugar was observed in addition to glucose and fructose after 8 hrs. of incubation and onwards thus showing the transglycosidase activity of invertase in *Blepharisma*. The hydrolysis of cellobiose and raffinose yielded glucose, and galactose and sucrose respectively thus showing the presence of β-glucosidase and α-galactosidase in *Blepharisma*. It seems β-galactosidase is absent as lactose was not hydrolyzed by *Blepharisma* homogenate. Since the hydrolysis

of raffinose did not yield any free fructose it seems that *Blepharisma* lacks a β-fructosidase also.

Proteinases

Both gelatin and egg albumin were hydrolyzed. The intensity of spots due to undigested protein was lowest between pH 5.8—6.6. Maximum hydrolysis of egg albumen occurred at pH 6.6.

Esterases

The homogenate did not hydrolyze either olive oil or ethylbutyrate since both controls and test mixtures maintained pink colour. Even the cytochemical tests using "Tween-80" as substrate (Gomori 1946) failed to show any lipase activity in *Blepharisma*.

Discussion

Carbohydrases

Amylase: Amylase has been studied in a number of ciliates (see Holz 1964). The pH optimum for α-amylase in *Blepharisma* was 6.0 (Fig. 1) like *Frontonia* (Vimala Devi 1965) and rumen ciliate *Epidinium* (Bailey 1958). However, it differs from that of *Stylonychia* and rumen ciliate *Isotricha* which have been reported as 6.8 (Hunter 1960) and 4.8 (Mould and Thomas 1958) respectively.

Glycosidases: The capacity to utilize oligosaccharides and polysaccharides has been shown in many ciliates e.g. *Paramecium, Colpoda, Tetrahymena* (Seaman 1955), rumen ciliates, *Isotricha, Dasytricha, Epidinium* etc. (see Holz 1964) and *Frontonia* (Vimala Devi 1965). However, different ciliates differ in their glucosidase activities. Unlike *Stylonychia* (Hunter 1960), *Blepharisma* resembles a few other ciliates in maltase activity e.g. *Tetrahymena* (Archibald and Manners 1959), *Frontonia* (Vimala Devi 1965) and rumen ciliates *Isotricha, Dasytricha* (Howard 1959), *Epidinium* and *Entodinium* (Abou Akkada and Howard 1960).

An interesting feature of the invertase of *Blepharisma* is its transglucosidase activity which results in synthesis of oligosaccharide in addition to glucose and fructose during hydrolysis of sucrose. Thus *Blepharisma* resembles other organisms like yeast (Edelman 1954), mold (Bealing 1953), siliate *Frontonia* (Vimala Devi 1965) and insects (Saxena and Bhatnagar 1961) in so far as transglucosidase activity is concerned.

Blepharisma resembles *Frontonia* (Vimala Devi 1965) and rumen ciliates *Dasytricha, Isotricha* (Howard 1959) in so far as the presence of β-glucosidase and α-galactosidase and the lack of β-galactosidase are concerned.

The proteinase of *Blepharisma* hydrolyzes gelatin at pH values between 5.8 and 6.6 and resembles that of *Tetrahymena* (Lawrie 1937) and *Frontonia* (Vimala Devi 1965). These three genera are further similar in hydrolyzing gelatin more readily than egg albumen. According to the classification of proteinases by Prosser and Brown 1961, proteinases of *Blepharisma* seem to include cathepsins.

Unlike *Tetrahymena* (Koehler and Fennel 1964), *Blepharisma* lacks lipid digestive enzymes.

The differences discussed above in the distribution and activity of various enzymes in different ciliates lend further support to Aldridge's 1953 statement "it is becoming clear that enzymes of the same type from different species may have a different spectrum of activities against a number of substrates".

Summary

The digestive enzymes of *Blepharisma intermedium* are described. The amylase of *Blepharisma* shows activity between pH 4.0—7.5, with a pH optimum at pH 6.0. Mercuric chloride inhibits amylase activity. The homogenate shows the presence of a maltase, an invertase of α-glucosidase type, α-galactosidase and β-glucosidase. The invertase shows trans-glucosidase activity. The homogenate seems to lack β-galactosidase and β-fructosidase.

The homogenate can hydrolyze egg albumin and gelatin. The maximum hydrolysis occurs between pH 5.8 and 6.6. Olive oil emulsion and ethyl-butyrate are not hydrolyzed by the homogenate of *Blepharisma*.

ZUSAMMENFASSUNG

Es werden die Verdauungsfermente von *Blepharisma intermedium* beschrieben. Amylase von *Blepharisma* wirkt zwischen pH 4.0—7.5, das Optimum ist pH 6.0. Sublimat hemmt die Aktivität der Amylase. Homogenat zeigt die Anwesenheit von Maltase, von α-Glykosidaseart der Invertase, von α-Galaktosidase und β-Glykosidase. Invertase zeigt die trans-glykosidase Aktivität. Bei dem Homogenat scheint die β-Galaktosidase und β-Fructosidase zu fehlen.

Homogenat ist imstande das Eialbumin und Gelatin zu hydrolisieren. Die Maximalhydrolisierung findet zwischen pH 5.8 und 6.8 statt. Die Olivenölemulsion und Äthylbutyrat werden durch Homogenat von *Blepharisma* nicht hydrolisiert.

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The influence of ultraviolet irradiation (2537 Å) on infusoria *Colpoda maupasi*

Реакция инфузорий *Colpoda maupasi* на ультрафиолетовые лучи (2537 Å)

The problem of study of the organism and cell reactions to the extremal influences which may act upon organisms beyond the Earth — on other planets and in the cosmic space — involves a great interest in elucidating the resistability of *Protozoa* to the short-wave ultraviolet radiation (UV) at various stages of their life cycle. The ultraviolet radiation of sun shorter than 3000 Å nearly fails to penetrate across the earth atmosphere being retained mostly by the ozone layer. Therefore the resistability of earth organisms to the short-wave radiation depends in the first place on the physical and chemical properties of all cell substances of the organism which hamper the penetration of rays to the sensitive components of the cell. It depends also on the capability of the latter to repair the injuries evoked by UV rays. Evidently the resistability to rays in the spectrum site under study is connected with the primary properties of the given species organism independently of adaptation because this process had been absent in the natural conditions of our planet in the oxygen period of its history.

The majority of investigations concerning the action of UV radiation upon protozoa had been carried out on their active forms. However the high resistability of cysts to various extremal action allows to expect that cysts might be more resistant to UV rays as well. However the data concerning this problem are absent in the litterature.

In irradiation of the ciliate *Colpoda*, the cell reactions may be examined on various stages of the life cycle: on the active vegetative stage (trophonts), in the period of reproduction (reproduction cysts) and at various conditions of rest of the cell when the synthesis of nucleic acids is slow or absent. Moreover the comparison of reaction to UV rays in *Colpoda* with that well investigated in *Paramecium caudatum* and some other protozoa, allows to elucidate some more general regularities in the development of the UV rays injuries and in the subsequent restitution of the cell.

Methods

Clones of the soil ciliate *Colpoda maupasi* were cultivated in the mineral medium of Lozina-Lozinsky 1948 with addition of *Bacillus subtilis* suspension as food.

A definite number of active ciliates or reproduction cysts were introduced by means of capillaries and irradiated by a lamp BUW-15. The power of the dose was 1 200 erg/mm² in a minute. Variation of irradiation doses was attained by applying different time of exposure. Doses from 2 400 to 36 000 erg/mm² were applied. Irradiation was applied to trophonts on the second day after excystment from resting cysts, reproduction cysts, resting cysts of the 8th and 14th day after excystment and desiccated cysts of 14 days of age.

The resting cysts were obtained by keeping the mass culture of ciliates without changing their medium for 7 days, accepting that till the 7th day encystation is fully accomplished.

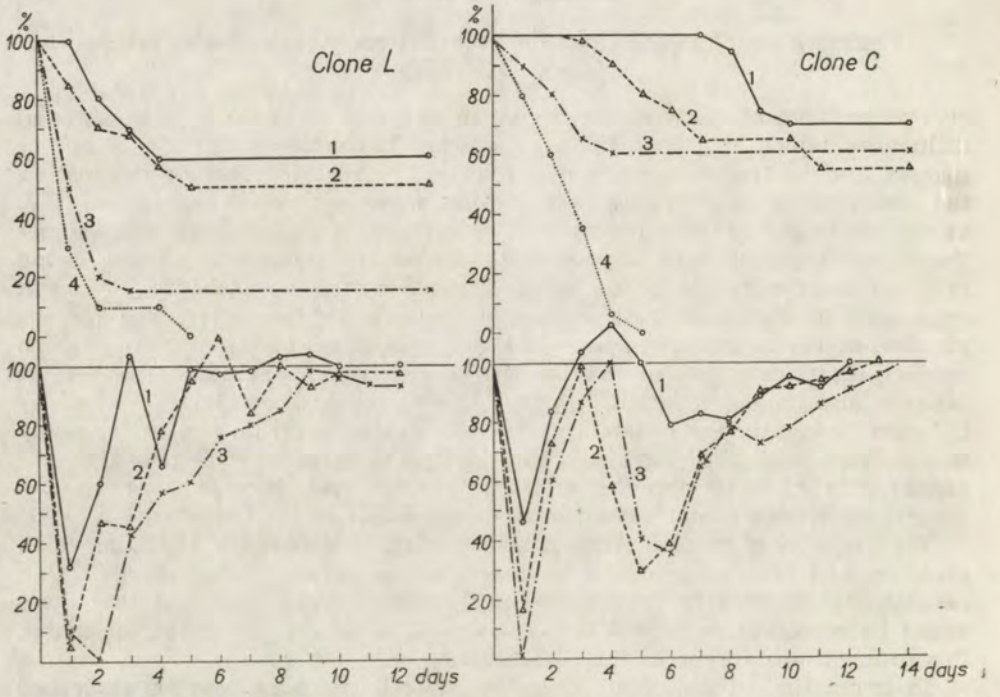

Fig. 1. Survival (up) and the division rate (down) of trophonts of clones L and C after UV irradiation with doses: 3 600 erg/mm² (1), 4 800—6 000 erg/mm² (2), 7 200 erg/mm² (3), 8 400 erg/mm² (4)
Abscissa — days of experiment, ordinate — survival (%) and division rate (%) to the control

Desiccation was carried out at room temperature in the course of 24 hrs. For dessication a definite number of resting cysts was placed in a container with fragments of cellophane with a small quantity of medium.

After irradiation, single ciliates were placed in microaquaria at red light and cultivated in darkness. In each experiment observations were carried out on 20 lines of irradiated and 20 lines of control ciliates.

The rate of reproduction was established after the number of ciliates

obtained from one individual as result of an every day separation. Observations were carried out in the course of 13—14 days. The survival of UV irradiated active cilliates was followed on two clones, and that of the cysts — on one clone L being more sensitive.

Results

Survival

The most sensitive proved to be trophonts — the active vegetative stage of Colpoda (Fig. 1). In on clone, LD_{50} was 6 000 erg/mm², in another one — slightly more than 7 500 erg/mm². A 100% death occurred at the dose 8 400 erg/mm². The reproduction cysts proved to be more resistant: LD_{50} for them as well as for the resting cysts 3 days of age, was 12 000 erg/mm² (Fig. 2). Excystment failed to occur after the dose of 16 800 erg/mm². After

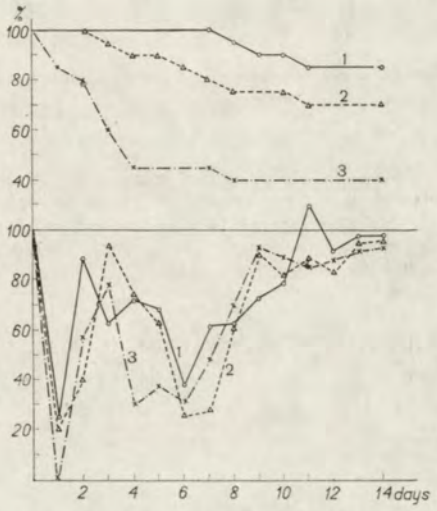

Fig. 2. Survival (up) and the division rate (down) of reproducting cysts after UV irradiation with doses: 2 400 erg/mm² (1), 9 600 erg/mm² (2), 14 400 erg/mm² (3) Abscissa — days of experiment, ordinate — survival (%) and division rate (%) to the control

a more prolonged condition of rest and especially after desiccation of cysts, their resistability becomes still higher: they stand (LD_{50}) such doses as 20 000 and 24 000 erg/mm² and perish entirely only after the doses higher than 30 000 erg/mm² (Fig. 3).

Comparing with the sensitivity to UV of other ciliates *(Paramecium caudatum)*, we observe that *Colpoda maupasi* is more resistant. For *Paramecium caudatum*, doses from 3 500 to 6 000 erg/mm² are lethal (Lozina-Lozinsky 1960, Samoilova 1964). The lethality of colpoda after irradiation embraces several days, sometimes up to 9—10 days. The highest percentage die on the first days. The higher the dose, the shorter the period of lethal exposure. The second rise of lethality which is observed in paramecia

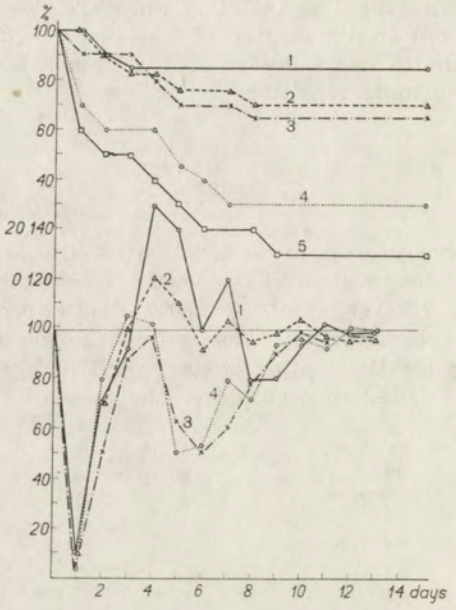

Fig. 3. Survival (up) and the division rate (down) of resting cysts of 14-day old after UV irradiation with doses: 7 200 erg/mm² (1), 12 000 erg/mm² (2), 16 800 erg/mm² (3), 24 000 erg/mm² (4), 28 800 erg/mm² (5)
Abscissa — days of experiment, ordinate — survival (%) and division rate (%) to the control

in the period of conclusion of the lag-phase, is absent in colpoda. In Fig. 4, survival of *C. maupasi* is represented at different stages of life cycle after irradiation with various doses of UV.

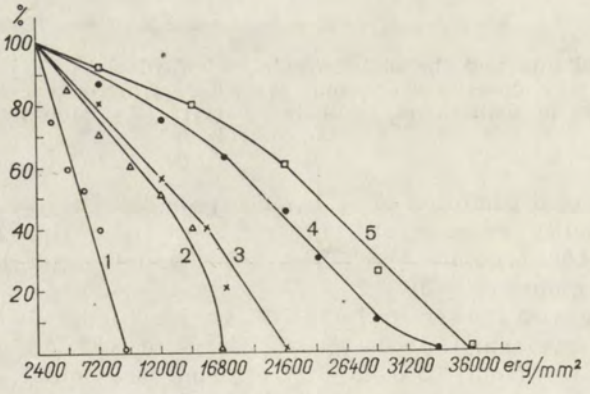

Fig. 4. Survival of *Colpoda maupasi* UV irradiated at the different stages of life cycle: trophonts (1), reproducting cysts (2), resting cysts of 8-day old (3), resting cysts of 14-day old (4), desiccated resting cysts of 14-day old (5)
Abscissa — doses (erg/mm²), ordinate — survival (%) to the control

The influence of different doses of UV irradiation ($\lambda = 2\,537$ Å) upon the rate of division.

The division rate in *Colpoda* after UV irradiation of active forms or cysts has not been studied till the present time. Two clones were subjected to irradiation, beginning with doses which evoke a retain of division on the first day after irradiation (2 min., or 2 400 erg/mm²) (Fig. 1). This dose — evidently the minimal one — is capable to retain the division of trophonts; in a less sensible clone, 35% of individuals failed to divide within the first 24 hrs. and in a more sensible one — 65%. In the first clone after a dose 4 800 and 6 000 erg/mm², and in the second one after 2 400 and 3 600 erg/mm², the rate of division regained its norm (control) on the 3—4th day. In a more sensible clone after the UV doses cited above, no secondary retaining occurs similar to that which is observed in paramecia (Alexandrov 1948, Lozina-Lozinsky 1960, Samoilova 1963 and others), and ciliates start dividing at the same rate as the control. At high doses — 4 800 to 6 000 erg/mm², the first period of the lowered division rate after irradiation continues without interruption till the middle of the 7th day. It may be postulated therefore that the first period of illness coalesse with the second one. However in this case, a typical lag-period is not observed because there is no full stop of division for several consecutive days in any of the lines. In the course of 24 hrs., ciliates undergo two or even three division cycles having 2—4 tomites in one reproduction cyst. As result, one ciliate often produces 16 individuals within 24 hrs. In the other cases in the irradiated ciliates one division cycle arises with 2—4 individuals in the course of 24 hrs, or one and a half of cycle with 6 individuals. Some ciliates which stopped their reproduction at once after irradiation, fail to recover and die.

In the more resistant chlones the picture of the UV illness resembles more to that which is known for paramecium. In this clone at the doses 4 800—6 000 erg/mm² after the first period of division retention, occurs restitution and subsequently the division rate becomes lower (compared with the control) for 4—8 days. In this case no full stop of division for a long time is observed in any line. High doses are lethal already in the first days after irradiation of trophonts and reproduction cysts, they therefore interfere with the possibility of lag-period.

It may be postulated that irradiation of trophonts in the periods between the reproduction cycles fails to impair the life stages and reproduction cycle phases which are sensitive in ciliates. In paramecia, owing to lack of synchronization in the development, individuals at different stages of cell cycle — even at most sensitive periods — might be irradiated and as result — a prolonged lag-phase occurred. Therefore after UV irradiation of reproduction cysts another reaction may be expected than in trophonts. In the experiments with irradiation of reproduction cysts of *C. maupasi* six doses were applied in the limits from 2 400 to 14 400 erg/mm². Immediately after irradiation of reproduction cysts, beginning with the lowest dose, division is stopped (Fig. 2). It restores for 2—3 days, and within 5—6 days a fall of the division rate occurs again. On the 7—8 day a secondary restitution of the reproductive functions follows which gradually reaches its normal state within 3—4 days. A typical lag-phase is also absent after irradiation of reproduction cysts as well even after maximal doses which evoke 100% of lethality. In this way, after irradiation of reproduction cysts when division takes place, the second

period of division retention is expressed more distinctly than in trophonts, the fall of division rate being only slightly associated with the irradiation dose.

In the second period, the illness after irradiation of resting cysts at the 8th day of age is far less acute than in the reproduction cysts. 14th-day old resting cysts are still resistant in respect of the subsequent reproduction and especially of their survival. Irradiation of cysts with doses from 7 200 to 12 000 erg/mm² not only fails to lower the division rate but stimulates reproduction even on the 4 and 5 day after irradiation (Fig. 3). A fall of the reproduction rate is observed 5—7 days after such doses as 16 800—24 000 erg/mm². 14-days cysts, when desiccated and irradiated subsequently with all the doses from 7 200 to 28 800 erg/mm², do not divide in the course of two days after transition to the active state which is connected with inhibition of excystment by desiccation. Consequently the disturbance of reproductive function in the first period after irradiation of desiccated resting cysts is the same as in the case of irradiation of reproduction cysts and trophonts. However the second step of illness is characterized by a secondary retardation of division which is expressed much less distinctly even in the case of such significant doses as 21 600 —28 800 erg/mm².

It is interesting that all the doses beginning with 4 800—6 000 erg/mm² evoke the same almost immediate effect of lowering the rate of division in 80—90% of all the forms of life cycle.

Photoreactivation

Trophonts and resting cysts 14 days of age were subjected to photoreactiv-

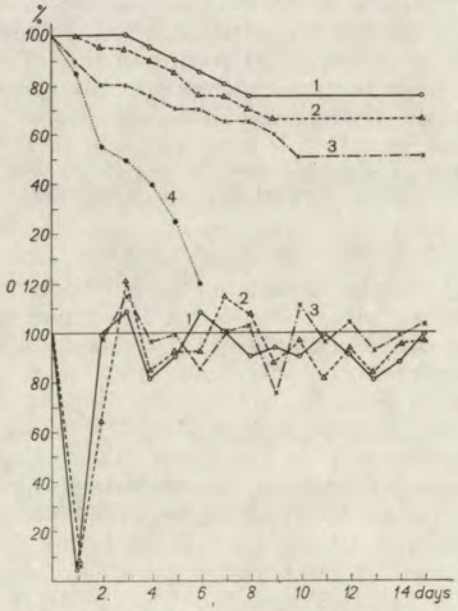

Fig. 5. Survival (up) and division rate (down) of resting cysts of 14-day old after UV irradiation with doses: 12 000 erg/mm² (1), 16 800 erg/mm² (2), 24 000 erg/mm² (3), 28 800 erg/mm² (4) and photoreactivated (PR)
Abscissa — days of experiment, ordinate — survival (%) and division rate (%) to the control

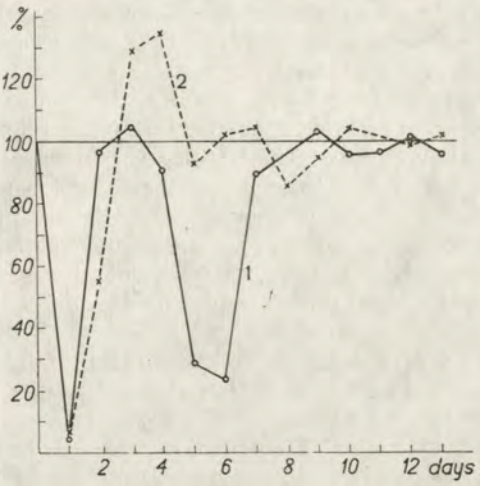

Fig. 6. The rate of division of trophonts after UV irradiation (7 200 erg/mm²), and photoreactivation: 1— UV without photoreactivation, 2 — UV and photoreactivation
Abscissa — days of experiment, ordinate — division rate (%) to the control

ation. After the action of the visible light the second period of fall of the reproduction rate is absent even after irradiation with high UV doses (Fig. 5, 6). The visible light fails to exert influence on the first stage of division retention.

Discussion

The study of reactions to UV rays in such an organism as *Colpoda maupasi* in which different stages of the life cycle are present (reproduction cysts, active vegetative stages and rest cysts), permitted to reveal the interdependence between the separate cycle stages and sensibility to UV rays as well as the capability to recover after irradiation. Moreover it was demonstrated that the reaction to UV rays in *C. maupasi* slightly differs from that in *P. caudatum* and in some other protozoa and animal cells. As known in paramecia, irradiation (even in comparatively low doses) is followed by a prolonged period of retention of reproduction (lag-phase) which attains 4—14 days (Lozina-Lozinsky 1960) and even 18—21 days (Alexandrov 1948). Consequently there are evident different factors which condition division at once after irradiation and the subsequent cellular cycles as decribed first by V. J. Alexandrov (1948), as the phenomena of UV impairment of two cell mechanisms.

In paramecia, besides the two periods of lowered division rate or its cease, occur two periods (peaks) of a raised mortality which are described for the cells of other organisms as well (Samoilova 1967). One of them coincides with the period following immediately after irradiation, another one with a later period of conclusion of the sickness. It indicates the deficiency of repairing possibilities in the cell. However sometimes the cells perish in the case of full restitution of division function (Lozina-Lozinsky 1960).

This indicates a great complexity of factors of the lethal effect after UV irradiation and the impossibility to reduce its action to impairment of two mechanisms. The experiments with *C. maupasi* indicate a sligthly different picture of the UV impairment of the cell and of the possibility of its restitution which may be summarized to the following phenomena:

1. In *Colpoda* the reproduction is fully inhibited after irradiation in the majority of lines, while it is lowered in the others being however restituted after one or two days. Both processes only sligthly depend on the applied dose and are observed in ciliates at all the life cycle stages. The sensibility of the mechanism responding at the first phase of illness, is considerably higher in *Colpoda* than in *P. caudatum* in which it depends at a high degree on the dose.

The suggestion that inhibition of the first post-irradiation division was the result of a general non-specific impairment of the cell (Alexandrov 1948, Lozina-Lozinsky 1960, Samoilova 1967) is evidently not sufficient for explanation of this effect because it scarcely depends on the dose and appears as well in the active forms as in the cysts which — as known — are much more resistant to the action of different external factors. It may be postulated that immediately after irradiation, an inhibition of enzymes occurs on which the release mechanism of the mitotic cycle depends. This mechanism is very sensible to UV rays. At last, against the hypotesis of a non-specific reaction expressed in the inhibition of division, speaks the normal state of *Colpoda* cells after irradiation with low doses. This does not exclude however the fact that a non-specific reaction to UV impairment exists. It increaeses with the dose and is manifested not only in a gradual depression but also in the fall of survival. This is indicated by the greatest resistability of resting cysts of 14-days age and of the desiccated ones.

2. The first period of illness in *C. maupasi* and in *P. caudatum* is accompanied by mortality. In *Colpoda*, the phenomena of division inhibition and of death proceed independently from one another: the death of separate lines occurs in the moment of an intense division and after its restitution with no symptons of depression which is characteristic for reaction of non-specific nature to the noxious agent e.g. to temperature, ionizing irradiation, chemical substances.

Mortality of irradiated *Colpoda* reaches usually its maximum in the first 24 hrs. and continues in the course of several days (up to 10 days) after the maximal doses but fails to attain a second peak as in *P. caudatum*. The evidence of non-specifity is expressed in the dependence of the stability of the survival test on the dose and on the stage of the life cycle.

3. In *C. maupasi* even in the case of lethal doses, the lag-phase is absent and only diminishing of the cell cycle number in 24 hrs. is observed. Inhibition of division depends at some degree on the dose and on the life cycle stage; the fall of divisions of individuals gained from the irradiated resting cysts requires a dose several times stronger than for an analogical division inhibition in the case of irradiation of reproduction cysts and trophonts. The period of restitution of the reproduction rate is not accompanied by the rise of mortality as it occurs in paramecia.

4. In *C. maupasi*, even a more delayed mortality is absent, being observed in paramecia subjected to UV and ionizing reaction and after the period of their restitution. This mortality is evidently connected with irreversible

changes in the genetic apparatus of the cell (Lozina-Lozinsky i Alexandrov 1959, Lozina-Lozinsky 1960).

Concerning the cytological changes evoking mortality and disturbance of the cellular division, we intend to compare those phenomena in the ciliates *Paramecium* and *Colpoda* only. Evidently it should be accepted as firmly established that inhibition of the first division and fall of the division rate in the first period of illness after UV irradiation, should be connected with the cytoplasmic impairment in the ciliate cell. This is supported by the fact of a rapid liquidating inhibition of the first division in *Colpoda* after its irradiation at different stages of life cycle. The disturbances arising in this case are nearly independent of the irradiation dose which is not characteristic for disturbances in the nucleic acids synthesis. Besides, according to the results of Samoilova i Ovchinnikova 1963, and Samoilova 1964 in paramecia, in the first period of illness, a fall of the cytoplasmic RNA is observed and some increase of the quantity of nuclear DNA. However evidently this is not the cause of mortality of a part of ciliates and of a short-lasting delay or stop of division. Moreover this stage of illness is not abolished by the action of visible ligth and — as known — the ligth rapairs the injuries connected with the nuclear apparatus and disturbance of nucleic acids synthesis in paramecia (Brand and Giese 1956, Samoilova 1964), i.e. disturbances which are characteristic for the second stage of illness (Lozina-Lozinsky 1960). In the initial period after irradiation, a significant impairment of mitochondria occurs as it was described in paramecia by Mashansky i Samoilova 1964.

A socondary fall of division rate and of the lag-phase in paramecia, depend possibly on a deep disturbance of RNA and DNA synthesis and on the changes occurring in the nuclei. Samoilova i Ovchinnikova 1963 have demonstrated that RNA synthesis stopped prior to the death of paramecia. The high resistability of resting cysts in the second period of illness and even stimulation of the cell division depend evidently on a low activity of nucleic acids in the rest period, however it may be associated with a lower permeability of cysts to the UV rays involved by the compactness of the cyst envelope.

The degree of disturbance of nucleic acids synthesis and the impairment of the nuclear apparatus under the action of UV rays is in *C. maupasi* — evidently — much lower than in paramecia. Photoreactivation in *C. maupasi* concerns only the second step phase of fall of the division rate and fails to raise the survival as it is observed in paramecia. Consequently, the recovery of ciliates which were not subjected to the action of light after irradiation as well as photoreactivation proceede in conformity in both genera. It may be postulated that photoreactivation and the "darkness" reactivation [1] are based on a similar physiological mechanism: the ligth strengthens the restitution, presumably by mediation of the cytochrome system (Lozina-Lozinsky i Zaar 1963, Muhammed 1965) which is possibly necessary for repa-

[1] "Darknesss" reactivation or reparation is not an appropriate term because it implies any mechanisms of cell restitution after irradiation, among other the ionization not connected with the action of light. However "darkness" does not belong to those agents. Possibly the restitution "darkness" occurs simultaneously with the action of light.

ration in absence of ligth. Cytochromes are indispensable for restitution of the yeast cells in darkness (S a m o i l o v a 1967). However the picture may be much more complex as it often occurs.

Summary

The resistability of infusoria to short-wave light of ultraviolet irradiation (UV) has been investigated in connnection with the problem of life beyond the Earth. The experiments were carried out on the soil infusoria *Copoda maupasi* at various stages of their life cycle. The survival and the rate of 14 days) the percentage of mortality decreases.

After UV irradiation ($\lambda = 2537$ Å) a delay of the first division of *C. maupasi* may be revealed, the duration of this delay being slightly depencent on the dose. After the recovery, the second delay of cell cycles takes place and lasts for 2—6 days. There occurs no complete cessation of division at this period even under the highest lethal doses. The highest percentage of death was found to occur on the first day after irradiation, in the later periods (up to 14 days) the percentage of mortality decreases.

The damages of different cell components evoked by ultraviolet rays result in decrease of the division rate and in lethality. According to the function of reproduction the reproduction cysts are more sensitive to UV than trophonts, the resting cysts being the next. The trophonts are more sensitive to lethal action of UV irradiation, than reproduction cysts. The resistability of resting cysts is many times higher than that of trophonts. The desiccated resting cysts show the highest resistability.

The visible light eliminates the second inhibition of division but it has no influence on the first period of it and on the rise of survival. The problems concerning the mechanism of injury and of recovery of cells are discussed.

РЕЗЮМЕ

Исследования проводились на почвенной инфузории *Colpoda maupai* на разных стадиях ее жизненного цикла (трофонты, репродуктивные цисты и цисты покоя). Изучалась выживаемость и темп размножения в зависимости от дозы облучения.

У *C. maupasi* после облучения УФ ($\lambda = 2537$ Å) наблюдается задержка 1-го деления, величина которой мало зависит от дозы. На 2—3-й дни происходит восстановление деления, а затем вторичное, менее сильное, торможение клеточных циклов. Это торможение функции деления не сопровождается полным его прекращением даже при самых высоких дозах, вызывающих значительную смертность. Вторичная реактивация деления протекает независимо от первой и продолжается от 2-х до 6-ти суток.

Смертность у *C. maupasi*, вызываемая большими дозами УФ, наиболее значительна в первые дни после облучения, затем постепенно снижается. Отдалённой смертности и её второго подъёма в период вторичного спада темпа размножения, наблюдающихся у парамеций, у *C. maupasi* нет.

Понижение темпа размножения и смертность вызываются повреждением УФ лучами различных механизмов клетки.

Судя по нарушению функции деления, наиболее чувствительными к УФ лучами являются цисты размножения *C. maupasi*, затем трофонты, хотя раз-

личия между ними в этом отношении не велики, и, наконец, цисты покоя. К летальному действию УФ лучей наиболее чувствительны трофонты, затем цисты размножения. Цисты покоя обладают значительно большей устойчивостью, которая увеличивается с их возрастом; максимальной устойчивостью обладают высушенные цисты покоя.

Видимый свет снимает торможение деления у *C. maupasi* во второй период УФ заболевания, но не влияет на первый этап задержки деления и на выживаемость. Облученные цисты покоя фотореактивируются.

Обсуждаются проблемы, касающиеся механизмов повреждения и восстановления клеток при действии УФ облучения.

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Fasciculi praeparati:

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