

MODES AND FORMS
OF
REPRODUCTION OF NEMATODES

BY
E. MAUPAS

Curator of the National Library of Algiers

Translation from the French: Marie-Anne Félix

INTRODUCTION

Nematodes reproduce and multiply solely by oogenesis. No case of scissiparity nor of blastogenesis is known at present among them¹.

For a long time, it was believed that their eggs were always and exclusively the product of the participation of distinct females and males, in other words that dioicy was among them the absolute rule.

This belief was actually the necessary result of the historical development in the study of these animals. For many long years, indeed, researchers have mostly dealt with parasitic species, then with marine species. In both of these groups, of which a large number of representatives are already known, separation of sexes appears to be an almost absolutely constant rule.

As far as marine species are concerned, all authors who made them the subject of their research, Ebhert (1863), Bastian (1865),

¹ The singular phenomenon of extroversion of the genital organs of the female of *Sphaerularia bombyi* and the hypertrophic development that they undergo following this hernia have nothing in common with gemmiparity, as Schneider believed it to be (*Monographie der Nematoden*, 1866, p. 322).

Marion (1870), Bütschli (1874), assume this dioicy for granted without letting appear the least doubt. This conviction is so well established that Marion, never having been able to encounter a single male individual in some species, whereas he often observed plenty of females, adds: "It may be unwise to give too much importance to these observations ¹". It is however quite likely that these species were either hermaphroditic or parthenogenetic. De Man, the most recent among these researchers and at the same time the one who saw and described the greatest number of species, summarizes his observations ² by stating that he always observed the presence of the two sexes.

The parasitic species, which are studied much more because of their large size and above all because of their practical significance, have for a long time confirmed this viewpoint. In all of them, distinct males and females were always found.

However, a first exception to this rule was already pointed out in 1860 by Schneider. At this time ³, indeed, he described under the name of *Pelodytes hermaphroditus* a small nematode discovered on putrefying matter. He watched this nematode, which was clearly female in aspect and organization, reproduce over a long succession of generations, without ever observing a male. By observing its genital organs, he could ascertain that once in the adult stage they started to produce a certain quantity of sperm, which was stored in a deep part of the uterus. By penetrating this latter organ, the eggs, which were formed later, were fertilized by these sperm. The German scientist had thus discovered the first case of protandrous

¹ *Annales des Sciences Naturelles: Zoologie*, t. XIII, 1870, article 14, p. 7.

² *Mémoires de la Société zoologique de France*, t. VI, 1893, p. 123.

³ *Zeitschrift für wiss. Zoologie*, t. X, 1860, p. 178.

autogamous hermaphroditism, of which we will see a multitude of examples in the following work¹.

A few years later², Leuckart, while studying the very curious biology of *Ascaris nigrovenosa*, demonstrated the absence of males in the parasitic form that lives in the lungs of batrachians. Because he had not recognized the presence of fertilizing elements in the genital organs of this parasite, he first³ stated that its eggs developed without fertilization and were therefore parthenogenetic. Later⁴, in the light of more exact observations by Schneider⁵, he recognized the existence of spermatozoa in the posterior of the uterus of the nematode. The parasitic *Ascaris nigrovenosa* was a second case of protandrous autogamous hermaphroditism.

Leuckart⁶, following Bastian⁷, also proposed parthenogenesis for the Filaria of Medina. These scientists relied on our complete ignorance regarding the males and on the absence of vulva and vagina in adult females. However, more recent observations⁸ seem to demonstrate the existence of males, the mating of which may even have been ascertained.

Around the same time, Perez asserted¹ the existence of reproduction through parthenogenesis in his terrestrial Anguillula. In one of his cultures, he saw Rhabditis females reproduce for several generations without having ever encountered a male. He concluded that his terrestrial Anguillula made use of facultative

¹ Later (*Monographie der Nematoden*, 1866, p. 315), Schneider changed the name of his *Pelodytes hermaphroditus* to *Leptodera fœcunda*. Today this species should be classified in the genus *Rhabditis*.

² *Nachrichten von der Königl. Gesellschaft zu Göttingen*, 1865, p. 227. – *Archiv für Anato. und Physiol.*, 1865, p. 641.

³ *Die menschlichen Parasiten*, t II., 1st fascicle, published in 1866, p. 61 and 148.

⁴ Id. 3rd fascicle, published in 1876, p. 691.

⁵ *Monographie der Nematoden*, 1866, p. 317.

⁶ *Die menschlichen Parasiten*, t II., 1866-76, p. 61 and 691.

⁷ On the structure and nature of the Dracunculus, *Transact. Linnean Soc.*, t. XXIV, 1863, and On the anatomy and physiology of the Nematoids, *Philosophical transactions*, 1866, p. 609.

⁸ RAILLET. – *Traité de Zoologie médicale*, 2nd edition, 1895, p. 502.

parthenogenesis, in which only females were generated, whereas fertilization was required to produce males. Unfortunately, Perez had confused several species in his work. The main object of his studies corresponds to the *Rhabditis teres* of Schneider. It is very common, and the many observers who studied it always saw it multiply through dioicious gametogenesis. As for the species without males that sprung up unexpectedly in his cultures, was it parthenogenetic, or else autogamous hermaphroditic? It is impossible to tell today, because Perez did not provide us with any anatomical or descriptive details about it. However, since we have encountered more hermaphroditic than parthenogenetic *Rhabditis* in our own research, it is highly probable that it belonged to the former category.

Around the same time, Schneider published his beautiful monograph on nematodes², in which the existence of protandrous hermaphroditic species was again claimed with the requisite demonstrative apparatus. In the course of his laborious research, this German scientist had often encountered species of *Rhabditis* (*Pelodera* or *Leptodera*) in which all individuals were females according to their external characters and internal organization. No male was ever found with these females. He believed he could distinguish seven distinct forms; but since in his opinion the species of *Rhabditis* were only well characterized by the shape of the male tail, he did not attempt to describe or define clearly these species without males. He named only four: *Leptodera dentata*, *dolichura*, *fœcunda* and *uncinata*, the head and tail of which he represented on his plates X, fig. 7, 10, 11 and pl. XI, fig. 7. Following Bütschli, we consider that Schneider's cautiousness was exaggerated and that the

¹ *Annales des Sciences Naturelles; Zoologie*, t. VI, 1866, p. 298.

² *Monographie der Nematoden*, 1866, p. 315 and following.

species in the *Rhabditis* genus can still be distinguished using the females, as long as they are carefully described and studied.

In any case, Schneider made long-term cultures of those species without males and saw them reproduce for many generations. Moreover, in all of them he observed the presence of spermatozoa in the uterus and, by observing larvae reaching the adult stage, he saw the genital gland produce first spermatozoa, then oocytes, the latter being fertilized upon their arrival into the uterus by the spermatozoa that were stored therein. All these *Rhabditis* were thus unquestionably protandrous autogamous hermaphrodites.

Schneider wonders if these hermaphrodites are able to reproduce themselves indefinitely through autogamous eggs. The fact appears to him improbable and he assumes that they undergo a heterogonic development, analogous to that described by Leuckart and himself in *Ascaris nigrovenosa*. The hermaphroditic generations must alternate with a dioicious generation, which appears in yet unknown circumstances and which is yet to be discovered¹. We do not share his opinion, and all our observations tend to make us believe that these hermaphrodites perpetuate themselves indefinitely by autogamy.

In addition to these *Rhabditis*, the German scientist also acknowledged the hermaphroditism of a *Dorylaimus* (*Enoplus liratus*) and, as said above, was the first to demonstrate the hermaphroditic condition of the parasitic generation of *Ascaris nigrovenosa*.

It is to Schneider again that we owe the first observation of a truly parthenogenetic species². Unfortunately, he did not describe

¹ Claus also argues for the necessity of a dioic form that would alternate with the hermaphroditic form. *Beobachtungen über die Organization und Fortpflanzung von Leptodera appendiculata*, 1869, p. 22.

² *Monographie der Nematoden*, 1866, p. 321.

nor illustrate this species, and only gave us some details about its genital organs. He saw it multiply without ever encountering males and without seeing the least trace of sperm in its uteri. Bütschli ¹ believes that he rediscovered the species of Schneider and described and illustrated it under the name of *Rhabditis Schneideri*. We know this species, having encountered it several times, and we believe the rediscovery by Bütschli to be perfectly exact.

A few years later, Vernet ² added to the list of hermaphroditic *Rhabditis* two species, in which he observed very precisely the early production of spermatozoa, followed by that of the eggs. He gave them the names of *Rhabditis terricola* ³ and *R. Leuckarti*.

The following year, Bütschli ⁴ again found the *Rhabditis dolichura* of Schneider and provided us with a good description, confirming the state of hermaphroditism that was pointed out by his predecessor. He further described, under the name of *R. Clausii*, a second species without males, the seminal reservoir of which was however always full of spermatozoa. He dared not commit to describing this species as hermaphroditic, although hermaphroditism is highly probable in this case. But his most interesting discovery is to have found again the parthenogenetic species mentioned by Schneider, which he described well under the name of *Rhabditis Schneideri*. Like his predecessor, Bütschli observed the complete absence of males and spermatozoa. We can confirm these observations, having verified them several times and,

¹ Beiträge zur Kenntniss der freilebenden Nematoden, 1873, p. 116.

² Archives des sciences physiques et naturelles. Genève, t. 145, 1872, p. 61-75, pl. I.

³ Vernet made a mistake by wanting to assimilate his first *Rhabditis* to the *R. terricola* of Dujardin. This latter species, according to its author, is a dioic *Rhabditis* that is therefore impossible to confuse with a hermaphroditic species. Therefore, the name *terricola* cannot be maintained for the hermaphrodite of Vernet, and I propose to dedicate it to the scientist who made it so well known to us, by calling it henceforth *Rhabditis Verneti*.

⁴ Beiträge zur Kenntniss der freilebenden Nematoden, 1873, p. 115-119.

again, we have in *Rhabditis Schneideri* the first well and duly established case of parthenogenetic reproduction in nematodes.

Parthenogenesis has been further demonstrated in a second species, although after long-lasting uncertainties and laborious researches. This case concerns the parasitic, or intestinal, form of *Strongyloides intestinalis*, this odd nematode with a heterogonic development, first found in its dioicious rhabditoid form in the feces of soldiers affected with diarrhea in Cochin China. Bavay, the first to describe the intestinal, or strongyloid, form had already noticed the absence of males. Grassi et Parona confirmed this first observation a little later and ascertained the absence of spermatozoa in fertile adult females. Meanwhile, Leuckart, having had the opportunity to study this parasite ¹, demonstrated definitively the alternating filiation between the two stercoral and intestinal forms, which were until then considered as two distinct species (*Anguillula stercoralis* and *Anguillula intestinalis*). The German scientist compared and likened this heterogonic development to that of *Ascaris nigrovenosa* and, chiefly guided by theoretical considerations, stated that the maleless parasitic form of the Strongyloid was protandrous hermaphroditic. The matter was only entirely clarified a few years later by Rovelli ². In a detailed and careful study of the genital organs of *Anguillula intestinalis*, this observer demonstrated the total absence of spermatozoa and consequently the development of the eggs through parthenogenesis.

Strongyloides longus, which lives in the small intestine of the rabbit, is parthenogenetic as well ³. Like its congener, it can be

¹ Ueber die Lebensgeschichte der sogenannten *Anguillula stercoralis* und deren Beziehungen zu der sogenannten *Anguillula intestinalis*. Ber. d. math.-phys. Classe der königl. sächs. Gesellsch. d. Wissensch. Leipzig, 1883, p. 85-107.

² Richerche sugli organi genitali degli Strongyloides. Como, 1888, II p. and I. pl.

³ B. Grassi and R. Segré. – Nuove osservazioni sull' eterogenia del *Anguillula intestinalis*. *Renconditi acad. Lincei Roma*, 1887, t. III, p. 100-108.

obtained in a rhabditoid form in cultures. But under this free-living form, all individuals, in an overwhelming majority (1000 females for 1 male), are ordinary females that remain sterile owing to male rarity. In the free-living form of *Strongyloides intestinalis* already, only one male is found for eight females.

We saw above that *Ascaris nigrovenosa* was a species with heterogonic development, whose parasitic form is hermaphroditic. This species was later placed in the *Angiostomum* genus together with four other species: *Angiostomum entomelas*, *rubrovenosum*, *macrostomum* and *sanguinolentum*. Linstow¹, having continued the study of these latter species, recognized that their development was identical to the first species, with a protandrous autogamous hermaphroditic state in the parasitic form.

Allantonema mirabile, which was so well studied by Leuckart², goes through a heterogonic development as well, with a protandrous hermaphroditic parasitic form.

We still have to cite a parasite of *Aphodius fimetarius*, i.e. *Bradyneuma rigidum*, whose protandrous hermaphroditism was elucidated through the work of Zur Strassen³. However in this case, in contrast to the previous species, the development is direct, as in the free-living hermaphrodites of the *Rhabditis* genus.

Oerley, in his monograph on the rhabditids⁴, enumerates four to five already known species that he acknowledges to be hermaphrodites, and then adds: "The greater part of nematodes have separate sexes, only a few are hermaphrodites, and parthenogenesis has not yet been well proven."

¹ Archiv für Naturgeschichte, 1885, t. I, p. 1-23.

² Abhandl. d. mathem.-phys. Cl. Königl. Sächs. Gesellsch. d. Wissensch. t. XIII, 1887, p. 505-704.

³ Zeitsch. F. Wiss. Zoologie, t. LIV, 1892, p. 655-747, pl. XXIX-XXXIII.

⁴ Die Rhabditiden und ihre medicinische Bedeutung, 1866, p. 37 and 58.

In summary, the well-established exceptions to the general rule are still quite few. At this time, one can estimate the number of known species of nematodes to about 1500 to 1600. Among these, only 18 were described as being able to reproduce without the help of distinct males. Of these 18 species, 15 are hermaphroditic and 3 parthenogenetic.

Here is the list thereof, with the names of the authors who first identified the true sexual state of these species.

Protandrous autogamous hermaphroditic species:

Rhabditis dentata, Schneider;

- *dolichura*, -
- *fœcunda*, -
- *uncinata*, -
- *Verneti*, Vernet;
- *Leuckarti*, Vernet;
- *Clausii*, Bütschli;

Dorylaimus liratus, Schneider;

Angiostomum nigrovenosum, Schneider;

- *rubrovenosum*,
- *entomelas*, Linstow;
- *macrostomum*, Linstow;
- *sanguinolentum*, Linstow;

Allantonema mirabile, Leuckart;

Bradyrnema rigidum, Zur Strassen.

Parthenogenetic species:

Rhabditis Schneideri, Schneider, Bütschli;

Strongyloides intestinalis, Rovelli;

- *longus*, Grassi and Segré.

These eighteen species can be divided in two equal halves between free-living and parasitic life. The nine parasitic species show a heterogonic development: they develop in a cycle of two alternating generations that differ in their form and organization. One of the forms spends its existence in the free-living state and always has two separate sexes; the other form lives as a parasite and is always hermaphroditic or parthenogenetic. The free-living species belong to the genera *Rhabditis* and *Dorylaimus*, the parasites to the genera *Angiostomum*, *Allantonema*, *Bradynema* and *Strongyloides*.

As can be seen in the above list, the *Rhabditis* genus provides the greatest share of species that reproduce without males. I do not believe that it should be concluded from these data that this peculiarity of organization is unique to this genus. *Rhabditis*, being easy to keep in methodical and numerous cultures, simply lend themselves better to this type of observations and were therefore the most well studied.

In the face of the well-established facts that we just enumerated in this historical review, it would seem that the notion of a reproduction without males, through hermaphroditic or parthenogenetic females, should have been accepted as a commonplace idea. It was however not so. The hermaphroditism and parthenogenesis of nematodes have entered science only with difficulty and even now are still sometimes dismissed as mistakes.

The very authors that discovered these facts appear to have only given them scarce attention, pointing them out as singular oddities rather than as phenomena of general interest. Schneider, who devoted a short chapter to them and to whom we owe the most numerous and most complete observations, does not even take the

time to describe with exactitude the animals on which he made his interesting discoveries.

The lack of interest given to these facts by their observers most clearly appears for those who provided us with the description of many species. They frequently describe species of which they only know the female, not having encountered any male. They satisfy themselves with mentioning this absence without comment and without trying to see whether it could correspond to a hermaphroditic or parthenogenetic state of the females. It seems that, in their mind, the lack of males simply and necessarily proves the greater rarity of this sex, which may sometimes be correct, but is certainly not so in all the many cases mentioned by these authors.

For example, Bastian, in his monograph on nematodes ¹, describes 58 terrestrial or freshwater species, among which 32 bear the note *male not seen*. Since then, males of 9 of these species have been found by de Man and by Bütschli; but 23 species still remain for which the male sex could not be found. A similar case exists for the 60 species described by Bütschli, in his work of 1873 ², among which 26 are only known through the females. Later, de Man described the males of 7 of these species, so that the number of species without any known male is reduced to 19. Nowhere in their text do these two authors appear surprised by this rarity and absence of males.

This state of the matter is perhaps even more apparent in the beautiful volume that de Man ³ devoted to the terrestrial and freshwater nematodes of the Netherlands. 145 species are described and illustrated with the most rigorous care and exactitude. Of this

¹ *Monograph on the Anguillulidae*, 1865.

² *Beiträge*, etc., 1873.

³ *Die Frei-Nematoden der niederländischen Fauna*, 1884.

number, 57 species come with the mention *unknown male*; which does not prevent the Dutch scientist from telling us (page 12): "All species that are described in my work have separate sexes; I have not encountered any hermaphrodite." He does not even consider the possibility of parthenogenetic species. Nonetheless, it is among his maleless species that we have recognized three of the forms with reproduction by parthenogenesis that we shall describe later.

In summary, the three works that we have just dealt with constitute to this day the three most important collections of species of nematodes. 206 distinct, well-described and illustrated species can be found therein. Of this total, 85, i.e. almost half of them, are only known through the females. It is probable and even certain that some of the missing males will be discovered; but we are equally convinced that, in many cases, they are normally missing, and that the future will show that we face well-established cases of hermaphroditism or parthenogenesis. We believe that here is a rich mine of interesting discoveries to be made.

If the specialists who made nematodes the particular object of their studies appear so hesitant about the problem of the sexuality of these beings, all the more reason for us to find the same uncertainty among the authors of general treatises. Thus, for Claus¹, nematodes have separate sexes, with the only exception of *Pelodytes* (*Leptodera fœcunda* of Schneider) and of *Angiostomum nigrovenosum*, which are hermaphrodites.

Taschenberg, in his excellent historical study on parthenogenesis², totally ignores nematodes.

¹ *Traité de zoologie*, 2nd French edition, 1884, p. 515.

² *Historische Entwicklung der Lehre von der Parthenogenesis*, 1892.

In the second edition of his zoology treatise, Railliet¹ simply writes: notwithstanding very rare exceptions (*Angiostomum nigrovenosum*), nematodes are dioicious.

E. Perrier² is more exact, since he admits as well established the possibility of parthenogenesis in some nematodes and cites the hermaphroditic *Rhabditis* of Schneider.

With Roule³ however, we fall back into almost utter doubt. According to him, except for some rare cases, the reality of which is doubted by several authors, unisexuality is the rule among nematodes. Roule uses the word unisexuality, because he only has hermaphroditism in mind and does not have the faintest suspicion of the possibility of parthenogenesis.

In summary, the problem of sexuality in nematodes has already been tackled several times; but it has always been laid out in such a vague and ill-defined fashion that nobody seems as yet to have perceived its true scope and great biological significance. Observations were gathered following chance encounters, without consistency and without method. The facts collected thereby have remained isolated and so insufficiently recognized that doubt could be cast upon them. The discovery of heterogony in the Angiostomes and Strongyloids has further contributed to divert the attention. This alternation of free-living and parasitic generations is undoubtedly very odd, but of limited biological significance. Nonetheless, a distinguished student of the most illustrious of modern helminthologists, dealing with these phenomena, could write⁴: "We know of a certain number of nematodes with protandrous hermaphroditism, but in all of them, the hermaphroditic generation

¹ *Traité de zoologie médicale et agricole*, 2nd edition, 1895, p. 389.

² *Traité de zoologie*, 1897, p. 1387.

³ *L'anatomie comparée des animaux basée sur l'embryologie*, t. I, 1898, p. 474.

⁴ ZUR STRASSEN in *Zeitschrift für wissenschaftliche Zoologie*, t. LIV, 1892, p. 730.

is parasitic and alternates with a free-living generation with separate sexes." He was thus completely ignoring the free-living *Rhabditis* species that are autogamous hermaphrodites, and far more interesting for general biology. Above all, these hermaphrodites and the parthenogenetic species are those that may shed some light onto the ill-understood role and significance of fertilization, as well as hermaphroditism and parthenogenesis, their origins, and relationships to dioicy. It is therefore to the study of these species that the present work shall be devoted.

METHOD

The nematodes that were used in our researches being unpublished species for the most part, we shall begin by describing them most accurately, placing them clearly in the framework of classification. These species almost exclusively belong to the rhabditid group (*Rhabditis*, *Cephalobus*, *Diplogaster*, *Plectus*) and especially to the *Rhabditis* genus. Schneider, as was previously said, was convinced of the impossibility of specifically differentiating the species of this latter genus without knowing the males. So he renounced describing the interesting hermaphroditic and parthenogenetic forms that he had encountered.

We do not share the opinion of the German scientist and we believe that with exact and careful descriptions, one can very well distinguish the species of *Rhabditis* that are only known through their females. We shall indeed see that the hermaphroditic species are not so completely devoid of the male sex as Schneider so believed, and that, when looking for it, one can always succeed in corroborating the specific female distinctions by the differential male characters.

Besides, these animals in many ways deserve the attention of biologists. Indeed, it is well known how important a role the Rhabditis type plays in the history of parasitism in nematodes. In the heterogonic species, one of the generations is always rhabditiform, and many of the migrating parasitic species also pass through a larval stage of the same type, before they reach their final conformation. It seems therefore almost indisputable that the Rhabditis were the stock from which many parasitic species are derived.

Moreover, they are not the least interesting for the sake of their biology. These species are much more numerous than is generally believed, and we have the certitude that unpublished species exist in greater number than those described to this day. We indeed possess in our notes twenty-three *Rhabditis*, eight *Cephalobus* and nine unpublished *Diplogasters*, even though we never particularly devoted ourselves to looking for new species.

But their chief interest for the biologist mainly resides in the ease with which these animals lend themselves to methodical cultures, which are easy to follow day after day. All species that we shall describe were thus placed in observation onto simple depression slides, which allowed to transport them at any time under the microscope. The slides were maintained in humid chambers, in order to prevent the evaporation of the few drops of water in which the nematodes were immersed. Their food, mixed with this water, could be dosed, renewed and varied at will. Most rhabditids multiply and grow very rapidly, so that the observer is able to see many generations pass under his eyes within a relatively short time. On the slides, they are isolated or raised in mass cultures, according to the need of the researches. The studies can be kept in warm incubators

or in cooling devices, so as to follow the effect of various temperatures. With these breeding methods, these nematodes thus become research subjects that allow the biologist to indefinitely combine and vary his experiments. With them, many important questions will be accessible to patient observers who do not fear long-term experiments. From this viewpoint, they can be compared to ciliated infusoria, the high biological significance of which we have elsewhere brought to light¹. With this method of breeding and long-term experimental cultures, these two types of animals complement each other: infusoria representing the elementary phenomena and functions of the cell, nematodes, the differentiated and condensed phenomena and functions of complex organisms. These considerations shall justify the care that we will take in distinguishing and making known the new and unsufficiently known rhabditids that served us in our studies.

RHABDITIS ELEGANS mihi

I came twice across this species in the surroundings of Algiers: a first time in May, a second time in November 1897. It lives in rich humus.

*Measurements*²:

| | ♀ | ♂ | YOUNG at hatching | LARVA encysted | ♀ out of the 4th molt |
|------|--------|--------|----------------------|-------------------|--------------------------|
| Body | 1700 □ | 1258 □ | 260 □ | 639 □ | 943 □ |

¹ *Archives de Zoologie*, 2nd series, t. VI, 1888, p. 178.

² The measurements of adults were always taken, when possible, on animals chosen among the largest individuals that we came across. The proportions 1/8, 1/10, 1/20, etc., placed in equivalence of the lengths of the esophagus, the tail and the diameter, express the ratio between these parts and the total length of the body. The length of the buccal cavity (vestibule) is always measured from the anterior end of the body, thus comprising the width of the lips. The esophagus measure is also taken from the anterior end of the body, thus including that of the buccal cavity. The equivalent proportion of the length of the buccal cavity gives the ratio between this length and that of the esophagus.

| | | | | | |
|-----------------|--------------|--------------|-------------|------------|------------|
| Esophagus | $200=^1/8.5$ | $186=^1/6.7$ | $83=^1/3$ | $150=^1/4$ | $185=^1/5$ |
| Tail | $160=^1/10$ | $36=^1/34$ | $58=^1/4.4$ | $80=^1/8$ | $131=^1/7$ |
| Vulva | 886 | | | | 514 |
| Diameter | $85=^1/20$ | $52=^1/24$ | $9=^1/39$ | $19=^1/33$ | $42=^1/22$ |
| Buccal cavity | $18=^1/11$ | $18=^1/10$ | $9=^1/9$ | $14=^1/10$ | $18=^1/10$ |
| Spicules | | 35 | | | |

The young females reach the size of 1,200 μ when the first eggs appear in the uterus. The size of adult females thus oscillates between this length of 1,200 μ and that of 1,700 μ .

The body has the shape (pl. XVI, fig. 1) of a very stretched spindle, which very regularly and gradually becomes thinner at both extremities. In the front, it is truncated by the buccal orifice; at the back it ends by narrowing in a tip of extreme fineness. When seen with transmitted light, it has a light greyish aspect, unlike the other Rhabditis, which always have an opaque blackish aspect. Later, when describing the intestine, we will explain the cause for this difference. The male (pl. XVI, fig. 2), always thinner and shorter, had a length in the individuals that I observed of 1,150 to 1,260 μ . It has the aspect of a slender filament with a diameter that is approximately constant along its whole length. The anterior extremity is truncated by the buccal orifice, the posterior one opens up in a wide bursa.

The cuticle, or external tegument, is colorless, rather thick and resistant. It is composed of two layers that are easy to separate. Indeed, letting the body of a Rhabditis that was killed by moderate heat macerate in 1 % acetic acid for half an hour is sufficient to see the external layer become bloated and detach from the internal layer (pl. XVI, fig. 3).

The external layer bears a fine transversal striation that is very difficult to see. There is also a very conspicuous lateral membrane in the shape of a small band. In the male, it shows a width of 5.5 μ in

the middle body region and thins out towards both ends, until it only forms a thin ridge in the esophagus region and the caudal region. At this latter end, it runs laterally without any relation to the bursa. In its enlarged region, one can easily distinguish four longitudinal and parallel striations. This band protrudes very little and represents an ornament, a superficial drawing of the cuticle, rather than a membrane in the true meaning of the word.

The lateral bands are very large, made of an amorph and nuclei-free substance. The dorso-ventral bands, on the contrary, are extremely narrow and reduced to simple lines at the point of contact of the muscle bands. The latter belong to the meromyarian type and are made of large spindle-shaped cells, which are nucleated and bear fine longitudinal striations. These cells can only be seen on emaciated individuals, after treatment with 2 % acetic acid.

The mouth (pl. XVI, fig. 4) ends the anterior extremity without being separated from the rest of the body by any narrowing or protuberance of any sort. It is lined with three little protruding lips that hollow out in their middle. Each of the lobes bears a papilla, the number of which around the full circumference of the mouth is thus six. – The buccal cavity, of 18 \square long, is wide and lined with thick walls. In the front it widens clearly; in the back, it is linked to the esophagus through the usual narrowings and thickenings.

The esophagus (pl. XVI, fig. 5), which in the females can reach a length of 200 \square , does not present anything noteworthy. The two bulbs are clearly marked. The posterior one, of an almost spherical shape, is provided with vigorous valves (teeth). In the front, the esophagus extends into a very visible thin sheath, which rises along the buccal cavity and envelops its posterior two thirds (pl. XVI, fig. 4).

The tail of females (pl. XVI, fig. 6) can reach a length of 160 μ . It is straight and, from its birth, becomes steadily thinner until it ends in an extremely fine tip. It bears a pair of thin lateral papillae, located slightly beyond the end of its anterior third.

The intestine, when seen with transmitted light, appears light greyish and never of the opaque blackish aspect that is so characteristic of most *Rhabditis*. This difference results from the fact that in *Rhabditis elegans* the cells of the intestine only contain albumino-fatty granules, and never the other type of granules that are very widespread in its congeners. These latter granules are characterized by their great birefringence and most likely represent regression products. Blackish and opaque in the transmitted light, they are the cause of the intense opacity of the intestine of many *Rhabditis*.

The intestine of our *Rhabditis* is composed of two rows of large alternating cells, the limits of which are however scarcely distinct, neither on the living form nor on prepared individuals. Their number and position can be ascertained after killing the nematode with moderate heat, then treating it with 2 % acetic acid and finally staining it with picrocarmine. After this treatment, large nuclei with a strong nucleolus can be distinguished very clearly (pl. XVI, fig. 1). In the females, there are 15 nuclei in each row, 30 altogether. In each row, 8 nuclei lay in front of the vulva, 7 behind. This number of 30 nuclei or cells is already present in the fourth stage larvae before the last molt, i.e. larvae that only measure 600 μ . Further growth is thus solely obtained through the extension of cells, without multiplication.

The young larvae that have just hatched (pl. XVII, fig. 3) and measure 260 μ , have 9 nuclei or cells in each row, 18 altogether. In

each row, 5 nuclei or cells are in front of the genital primordium and 4 behind. Of these 18 primitive nuclei or cells, 12 only will subsequently multiply by doubling during larval growth.

It was interesting to observe in this hermaphrodite an organization of the digestive tract similar to that of ordinary *Rhabditis*. Schneider, indeed, states ¹ that the intestine of the hermaphrodites that he observed was always composed of many small polyhedral cells, and he concludes that this constitutes an important distinctive character between the hermaphroditic *Rhabditis* and the dioicious *Rhabditis*. It is indeed certain that the intestine of one of the species from Schneider, *Rhabditis dolichura*, is composed of numerous small cells arranged in four rows. But I am convinced that this peculiarity bears no relation with the hermaphroditic state of this nematode.

The nerve ring has the usual shape (pl. XVI, fig. 5) and arrangement, and envelops like a tie the narrow part of the esophagus above the second bulb. On the ventral side, it extends obliquely backwards in the direction of the excretory pore.

The excretory apparatus is difficult to see well. The pore itself and a tiny chitinized canal can only be discerned at high magnifications. As to the lateral canals, they are to be observed on very emaciated and highly compressed individuals, with homogeneous immersion objectives. One can then follow the posterior branch almost until the rectum and dimly see the anterior branch, extending up forwards along the esophagus. The pore is located (pl. XVI, fig. 5) at the level of the second bulb.

The female genital apparatus (pl. XVI, fig. 1) is built according to the paired type that is usual in the *Rhabditis* genus. The vulva,

¹ Monographie, etc., p. 315.

located slightly behind the middle of the body, does not have protruding lips. The very short vagina is hardly marked. The uteri, on the contrary, are very large and each of them, in fully developed females, can contain as many as 25 to 30 eggs. The tuba, or oviduct, is compressed between the uterus and the large mature ova that fill the vitellogen. It has the shape of a simple tube, bent in S (pl. XVI, fig. 7). Its middle part, strongly inflated, serves as a seminal receptacle. Within the ovary, one can distinguish two regions: the vitellogen and the germigen. The vitellogen, which follows the oviduct, extends directly on the anterior side until a little before the second bulb; on the posterior side until a little before the beginning of the rectum. It always contains large ova in the process of growing and maturing. At the extremity of the vitellogen, the ovary bends suddenly in the opposite direction and extends until a little before the middle of the uteri. This curved part represents the germigen. It is composed of a well-developed central rachis, which is easy to see (pl. XVI, fig. 1) on individuals treated with 1 % acetic acid, and of a simple layer of germ cells (pl. XVI, fig. 8), which surround it like an epithelium. The whole is enclosed in a thin anhistic membrane, an extension of the uterus, of the oviduct and of the external membrane of the vitellogen. On prepared individuals, a few nuclei can be noticed within this membrane, as well as a small terminal cell at its blind end (pl. XVI, fig. 9).

The male tail opens up in a wide bursa (pl. XVI, fig. 10, A, B), which is very prominent, closed in the front, somewhat heart-shaped when seen fullface and completely enveloping the extremity of the tail. This bursa is provided with nine pairs of papillae, laid out by groups of three. The papillae are large and strong. Only two of them are preanal.

The spicules, of a length of 35 μ , are not very strong. They are of a rather dark brown color and are independent of each other. Their tip is very thin. The anterior extremity of the accessory piece, or gubernaculum, curves towards the ventral side inbetween the two spicules. The piece in its whole is slender and thin.

A rather strong rectal gland with a big nucleus is found on the dorsal side of the end of the intestine (pl. XVI, fig. 10, B).

The testis (pl. XVI, fig. 2 and pl. XVII, fig. 1) is built according to the usual type for this organ in the *Rhabditis* genus. It thus does not present anything especially noteworthy.

The spermatozoa are of a shape (pl. XVI, fig. 11) and structure that are absolutely identical in females and males. Spherical and of a small volume, they only measure 2.5 μ in diameter. Their cytoplasm, being very finely granular, allows to discern a small nucleus of oblong shape and of compact structure. I never observed the least sign of motility.

The eggs of this *Rhabditis* are not laid when arriving into the uterus, but on the contrary remain there for quite long and are stored in rather large numbers. In the large-size females, 25 to 30 of them can be counted in each uterus. It thus results, that with their rapid development, these eggs are always laid at a rather advanced stage of development and often hatch inside the uterus itself. The juveniles, at the beginning, are being expulsed with the eggs that have not yet hatched; but when the supply of sperm is exhausted and unfertilized eggs without a shell arrive into the uterus, the juveniles that are found there at this time feed on these eggs and grow in the uterus. Once they have become larger and stronger, they finally tear apart the walls of this organ and spread into the general body cavity. They then disrupt and devour the viscera of their mother, who soon

perishes. It is thus not rare to find, in the cuticular bag of the latter, individuals that fully developed therein and became adults. This species can therefore be said to be ovo-viviparous.

Several times, I isolated females in order to find out the number of fertilized eggs that they could lay. This number, in the most vigorous females, does not exceed 230 to 240, after which all produced eggs remain unfertilized and sterile. It must be thus concluded that the genital gland is limited to this figure of 240 spermatozoa in its function as a testis. Besides, this testis role must be very short and the production of spermatozoa very fast. Indeed, I saw, at a temperature of 20° C., a female that was isolated at the time of her hatching produce her first egg exactly 53 hours later. She must therefore have passed within these 53 hours through all the larval stages and produced her stock of sperm.

This figure of 240 spermatozoa is a maximum that is not always reached and can even be strongly reduced. I have indeed seen females, reared in bad conditions, produce only 30 to 40 spermatozoa.

The existence of this nematode is fast and short. At a temperature of 20° C., it takes three days to develop from fertilization of the egg to the adult stage, including 15 hours of embryogenesis inside the egg shell. During the following three days, it lays its 240 eggs by successive layings of 100, of 80 and of 60 eggs every twenty-four hours. Then, once it has exhausted its stock of spermatozoa, one of two alternatives may occur. Either it ejects the very last fertilized egg or juvenile hatched in the uterus, and in this case it will live for a further 4 to 6 days in a sterile state, laying now and then packages of unfertilized eggs, devoid of a shell and immediately falling apart. Or, one or several juveniles, having hatched in the uterus and starting

their first development by feeding on these unfertilized eggs that have accumulated in the uterus, will end up tearing apart the walls of this organ and spread into the general cavity. There, they will attack and devour the viscera of their mother, who soon will succumb and perish. In the first alternative, the longest lifespan will have been obtained; but altogether will not exceed a total of 10 to 12 days. In the second alternative, life will only have lasted for 7 days. It even often happens that intra-uterine hatchings result in the death of the mothers as soon as the 6th and sometimes the 5th day.

The juveniles that have just hatched (pl. XVII, fig. 3) measure from 250 to 260 μ . They already possess all their organs, except the genital apparatus, which is still in a very rudimentary state. But, as can be seen in the measurement table, the proportions of the body parts are very different from those of the adult state. The esophagus indeed equals $1/3$ and the tail $1/4$ of the total length, instead of $1/8$ and $1/10$. It thus results that in the final growth the greatest share is taken by the middle or intestinal region, which expands 11-fold, whereas the esophagus and the tail only expand 2 to 3-fold. The genital primordium (pl. XVII, fig. 3, g), located slightly behind the middle of the body, has an elliptical shape. It is made of two large germinal central nuclei, flanked at either end by a small somatic nucleus, the whole being enveloped by a thin anhistic membrane.

I did not observe the molts of this *Rhabditis*: the encystment has been described in my work on the molt and the encystment, on page 612¹.

This species is very agile. The animal wriggles and moves in a lively manner through undulatory movements similar to those of the eel. It is more agile than *Rhabditis dolichura*.

¹ Archives de Zoologie, 3^e série, t. VII, 1899.

Among the hermaphroditic *Rhabditis* that have been described to date, two fairly resemble *Rhabditis elegans*: *Rhabditis dolichura* and *R. terricola* (= *Verneti* mihi) of Vernet. It distinguishes itself from the former by its larger size, by its clearly developed anterior bulb, by its much shorter coecum, by its longer tail, by its ovo-viviparity, by the structure of the intestine and finally by the male bursa, as we shall see later when describing that of *R. dolichura*. The similarities with the latter are much greater. Indeed, there is identity in the conformation of the ovary and of the esophagus, the structure of the intestine, the ovo-viviparity, the length of the buccal cavity¹ and the length of the rectum. But *R. Verneti* differs by its notably larger size, by its less round first bulb, by the greater length of its esophagus, by the more anterior location of the excretory pore and by the squatter and shorter shape of the tail. Its eggs also appear slightly less voluminous. Its male is unfortunately not known.

Once I had discovered this species and recognized its hermaphroditic state, I cultured it on depression slides and multiplied it, so as to obtain a large number for studying it. Several thousands of individuals, all females, thus passed under my eyes. Only after about twenty days did I discover the first males.

In order to ascertain their frequency and their proportion, I organized special cultures. On a depression slide, I placed fifteen to twenty well-fed females and let them lay for twenty-four hours, then removed them. The juveniles from these layings were maintained together and fed well. Once they had become adults, I removed them with a pipette by batches of about hundred. These batches were

¹ In the text of Vernet, the buccal cavity (vestibule) is labeled, obviously by mistake, as having 28 μ in length; whereas if it is measured according to the drawing, executed at a magnification of 200 times, one finds a length of 17 to 18 μ . I obtained the figure for the magnification, which was not indicated by Vernet, by calculating it from the measurements and the drawings of the esophagus and the tail.

spread onto a slide in a long and thin band. Thus displayed, it was easy for me to examine these preparations with a low-magnification objective and extract the males from them, when they could be found. As to the females, since it was impossible to count them alive because of their great agility, I killed them by passing the slide over the flame of an alcohol lamp; I could then count them exactly.

These cultures and these operations, twelve times repeated, gave me a total of 20,032 individuals, among which 20,002 females and 30 males; that is to say 1.5 males for 1,000 females. This figure of 1.5 for 1,000 is an average number, taken from all my cultures. It is not rigorously exact for each of them. It can be higher. Indeed, in one of the cultures with 1,500 individuals, I found 9 males: that is to say 5.3 per 1,000. However, in four other cultures totalling 4,122 individuals, I did not encounter a single one.

These males, as we saw in the description of the species, are, as for all nematodes, thinner and shorter than the females. They are also much more agile and are seen to be constantly moving swiftly among the females. This perpetual agitation and this agility make them highly difficult to disentangle in the midst of the confused swarming of these little animals. All those that I have observed appeared to me to have a regular and perfect organization in all their parts. The bursa and the copulatory organs had a good constitution, and the testis as well (see pl. XVI, fig. 2 and pl. XVII, fig. 1). The seminal reservoir was literally crammed with spermatozoa, identical in their volume, structure and conformation to those produced by the hermaphroditic females.

When I had recognized the existence of these males, I sought whether they appeared in specific conditions that could be determined.

I wondered whether the age at egg-laying was a factor. A young age certainly did not contribute at all, since in my cultures I had always used females that had just reached the adult stage. I therefore wanted to verify whether this held true for the old age. I gathered and isolated 50 females that had almost exhausted their supply of sperm. All the juveniles that were produced in their last layings were kept and cultured. I obtained 244 individuals, which all, once at the adult stage, became females. The age at egg-laying had, therefore, no influence on male production.

I also tried whether food had any influence on this determination. It is indeed known that, for some authors, the matter of sexuality could be reduced to a mere problem of nutrition. According to these authors, the well-fed females would be thelytokous (producers of females), the ill-fed, arrhenotokous (producers of males). I experimented on *Rhabditis elegans* in the following manner: I started a culture with juveniles that I had let hatch in a pure water drop and gave them egg albumin as only diet. This nematode can live and grow with this nutrient; however, its development is far slower and, once at the adult age, it remains thinner and shorter. The females fed in this manner hardly lay a third of the eggs produced by individuals fed with rotting flesh. The albumin thus constitutes an inferior, less nutritious food. In this culture, 280 individuals reached the adult stage, among which no males was to be found. The bad nutrition thus had no noticeable effect on sexuality.

I had observed on several occasions that the hermaphroditic females that did not succumb to intra-uterine hatchings could still live for several days after having exhausted their own sperm and lay many eggs, which were necessarily unfertilized. These sterile eggs could be easily recognized by their incomplete organization, mostly

characterized by the absence of a chitinous shell; which resulted in them being seen to fall rapidly apart and dissolve into an amorphous gruel within the surrounding liquid medium.

I thought to make use of this peculiarity to try and refertilize by the males these females that had become sterile. I would then obtain eggs that would be the product of heterogamous fertilization and may perhaps behave otherwise as those from autogamous fertilization. In my cultures, I thus was careful to have at all times at my disposal females that had exhausted their own sperm and, each time I would encounter males, I would combine them together in special cultures, watched over and registered in a daily journal. All the females thus used had ceased to lay fertilized eggs since at least twenty-four hours.

I have organized thirteen such experimental cultures. The five first ones were made at the end of June and the beginning of July, at a temperature of 24° to 25° centigrade; the remaining eight, in the month of December with temperatures of 15° to 16° centigrade. In the daily journal, I wrote down the state of the animals, their deaths, their egg-layings, the number of laid eggs and the hatching of these eggs, the growth of the juveniles and finally their sexuality, once they had reached the adult state. I will not reproduce here all these details, which would lead us to useless repetitions. I will make do with summarising them in the following table with six columns. The first one gives the number of females in the experiment, the second that of the males, the third that of the laid juveniles, the fourth the number of females among these juveniles, the fifth that of the males, the sixth, finally, the length of the experiment expressed in days.

| | ♀ | ♂ | JUVENILES | ♀ | ♂ | DAYS |
|-------------|-----|-----|-----------|--------|--------|------|
| I | 11 | 7 | 11 | 8 | 3 | 6 |
| II | 8 | 7 | 18 | 8 | 10 | 5 |
| III. . . . | 10 | 3 | 96 | 51 | 45 | 4 |
| IV. . . . | 11 | 12 | 0 | 0 | 0 | 4 |
| V | 10 | 14 | 35 | 17 | 18 | 4 |
| VI. . . . | 18 | 5 | 45 | 26 | 19 | 6 |
| VII . . . | 12 | 4 | 69 | 37 | 32 | 5 |
| VIII. . . | 10 | 19 | 0 | 0 | 0 | 5 |
| IX. . . . | 5 | 11 | 0 | 0 | 0 | 5 |
| X | 11 | 10 | 0 | 0 | 0 | 7 |
| XI. . . . | 8 | 11 | 0 | 0 | 0 | 6 |
| XII | 9 | 4 | 0 | 0 | 0 | 9 |
| XIII. . . . | 16 | 5 | 0 | 0 | 0 | 9 |
| TOTALS . | 139 | 112 | 274 | 147 | 127 | |
| | | | | 53.6 % | 46.3 % | |

The fact that strikes one first at the sight of this table is the small number of refertilized females. Indeed, 139 females were brought in contact with 112 males, for a length of time that varied from 4 to 9 days; and nonetheless only six of these females were refertilized. I say that only one female was refertilized, even in experiments III and VII, that gave us the highest layings, taking as a reference the staggering of these layings, which occurred according to the usual course for a unique mother in this species.

During these experiments, I brought the preparations many times under the microscope in order to see what was occurring. Despite this, I only find in my journal three mentions of males directly observed to be mating. I saw them instead to be very active and very agitated, circulating without rest among the females, colliding into

them at every moment, but without appearing any more concerned about them than if they had been any inert bodies. The sexual instinct thus seems rather little developed in these males. But since there lies a highly interesting question, on which I shall have to return regarding the other species, I will here no longer insist on the matter.

Another fact, related to the previous one, is the absolute sterility observed in experiments IV, VIII, IX, X, XI, in spite of the fact that they were all provided with a higher proportional number of males than the others. Now, according to the notes in my journal, all males in these experiments were of heterogamous origin, whereas in all the other experiments, they had their origin in an autogamous fertilization. It thus seems to result from this fact that the heterogamous fertilization, far from regenerating the sexual energy and instinct of males that derive from it, seems rather to contribute to weaken them even more. I say seems because there were not enough observations to assert it fully. What is certain on the other hand is that these heterogamous males, in spite of their large number, are not of any more worth for the propagation and conservation of the species than the autogamous males. I actually doubt that any male ever occurs in nature. With animals so little lustful as the males of our *Rhabditis*, the very special conditions of sequestration in which I maintained them must be required to induce some of them to mate.

But the most important fact that is recorded in the above table is the high proportion of males obtained in the cultures originating from the heterogamous fertilizations. From 1 or 2 males per thousand, this proportion rises to 463 per thousand; i.e., the usual figure in dioicious *Rhabditis*. Following heterogamous fertilization,

the balance between both sexes is thus restored. The fertilization of these hermaphroditic females, which are almost fully thelytokous, by their extremely rare males has therefore a very pronounced arrhenotokous influence on them. Brauer ¹ had already noticed in *Apus* a similar arrhenotokous action of fertilization by males. According to him, when the parthenogenetic females of this phyllopod crustacean are fertilized by the extremely rare males, they cease to lay female eggs and only lay male eggs. Here arrhenotoky would have become absolute.

This arrhenotoky, caused by the fertilization of males of special origin, acting on females with an equally peculiar sexual organization, was very interesting to record. It is the counterpart to what happens in the bees, where fertilization determines an absolute thelytoky. It is a novel case of sexual determination that lies outside the influences of nutrition, which some authors claim to be the only ones to play a role in these phenomena.

The females of heterogamous origin, sisters of the males that we have just dealt with, are protandrous hermaphroditic females, absolutely identical to those of autogamous generations. I nevertheless wanted to ascertain whether the products of their layings were also similar, in other words whether the heterogamous fertilization would not retain some influence on the individuals of the second generation. I thus kept 38 females of heterogamous origin and let them lay, rearing until the adult stage the juveniles born of these layings. I thus obtained 2,964 individuals, among which 2,957 females and 7 males, that is 2.3 per 1000 males. This ratio is almost the same as that of the large cultures that were summarized above. It must be concluded that the effect of

¹ Beiträge zur Kenntnis der Phyllopoden. In: *Sitzungsberichte der kaiserlichen Akademie der Wissenschaften zu Wien*, t. LXV, 1872, p. 279-291.

heterogamous fertilization is not felt at the second generation anymore.

Below, once we have studied the other protandrous autogamous hermaphroditic species, we will develop the ideas that we reached concerning the significance of these rare males for the general biology of these beings.

Towards the end of the first series of experimental cultures that were undertaken in June and July, I once wanted to examine in the microscope, at a high magnification, the genital gland of a male. I was most surprised to see beautiful ova occupying the region of the gland where the spermatoblasts normally mature (pl. XVII, fig. 2). These ova appeared very well formed, of normal dimensions, with a large nucleolated germinal vesicle, and arranged in a regularly growing series. They were absolutely identical in all respects with the ova of the genital organ of females. The testis did not present anything peculiar in its general conformation and the germinal reservoir contained many spermatozoa, piled up together, as in all males observed so far. No doubt was possible: I had under my eyes a protandrous hermaphroditic testis, similar to the ovary of the sisters of this male.

At that time, I still had 20 males in my cultures. I examined each one of them and found 6 hermaphrodites and 14 unisexuals. These males all originated from heterogamous fertilizations.

In my second series of cultures, undertaken in the month of December, I sought to determine whether hermaphroditic males could be found again. To this end, I examined with the greatest care 60 males, 10 of which were of autogamous origin and 50 of heterogamous origin. When I placed them under the microscope, they had been adults for 4, 5, 6 and 7 days. I did not find ova in any

of them. These cases of male hermaphroditism thus do not appear to be very common.

It was interesting to experimentally ascertain whether the hermaphroditic generations could be maintained and reproduced indefinitely through simple autogamous fertilization; or whether their reproductive faculties would weaken and become exhausted by a strict autogamy, and whether a cross-fertilization would thus be required from time to time to regenerate them.

In order to solve these interesting questions, I organized a culture in which I always cared, at each generation, to isolate the reproducers before they became adults, so as to be very certain that no male would intervene in the fertilization of the females. Each generation was registered in a journal at the time when the first eggs were laid. Twenty reproducing females were isolated together at each generation. Their mixed eggs and the larvae that resulted formed the following generation.

This culture was maintained in this manner from the 1st of December to the end of June, and during this period of time, I recorded 52 generations. During the winter months, with an average temperature of 14° to 15° centigrades, the generations followed one another every five days. In the month of June, when the temperature rose to 23° or 24° centigrades, they recurred every other day.

During the months with a cool or average temperature, all representatives of a generation appeared vigorous and healthy. But when we reached the temperatures of 23° to 24° C. in the month of June, I noticed many cases of degeneration. Many eggs, after they had started to develop, were arrested at various degrees of development and finally aborted. In some of them, small larvae could be seen folded on themselves; but they were so weak and puny

that most of them did not even manage to break their egg shell. Those who succeeded, ill-conformed and unable to take any food, were not long to perish. These cases of weakening and abortion became so numerous in the last days of June that I had to renounce continuing this culture.

At first, I could have been tempted to attribute this degeneration to the reproduction by strict autogamous fertilization. But since I was simultaneously rearing another hermaphroditic *Rhabditis*, *R. Duthiersi*, placed in methodical cultures for some weeks only, and noticed in this culture even more numerous and intense abortions and degenerations, I had to rule out this explanation. I thus believe that the single and true cause of these degenerations was the rise in temperature. This opinion appears to me all the better founded since in the months of June and July of the previous year, I had already noticed similar phenomena in the cultures from which I drew the subjects for my refertilization experiments. I had indeed to stop this first series of experiments, following the impossibility of obtaining new subjects. And yet I had this nematode in culture for only two months, since I had discovered it on the 1st of May. The cause for these degenerations must thus indeed be attributed to temperature. But we shall return to this point concerning *Rhabditis Caussaneli*.

In any case, this culture demonstrated that this nematode could reproduce regularly for at least fifty generations with strictly autogamous fertilizations.

RHABDITIS CAUSSANELI mihi

I found this species only once in Vire, Normandy, in the month of September 1896. I had disemboweled an *Arion empiricorum*, variety

ater, to search in its intestine the nematodes that Dujardin and Schneider had reported. Instead of the latter, I encountered five encysted larvae, which I isolated on a depression slide with rotting slug flesh. These larvae desencysted, grew, and two days later, started to lay eggs that developed regularly. I was dealing with a new autogamous protandrous hermaphrodite. Since I had to come back to Algeria soon after, I provoked the encystment of the many descendants of the five first larvae by feeding them poorly. I deposited the encysted larvae thus obtained onto wet rich soil, which I enclosed in a large glass tube. Ten days later, once arrived in Algiers, I organized a terrarium with this Norman soil and placed some small pieces of rotten flesh on top. Two days later, I had many specimens of the new hermaphroditic *Rhabditis*, with which I organized cultures on depression slides, where I have maintained it for almost two full years. I dedicate it to the memory of the regretted Dr Caussanel, professor at the Medical School of Algiers.

Measurements:

| | ♀ | ♂ | YOUNG at hatching |
|---------------------|----------|----------|----------------------|
| Body | 3074 □ | 1973 □ | 300 □ |
| Esophagus | 343=¹/₉ | 286=¹/₇ | 89=¹/₃ |
| Tail | 128=¹/₂₄ | 57=¹/₃₄ | 33=¹/₉ |
| Vulva | 1544 | | |
| Diameter | 171=¹/₁₈ | 100=¹/₁₉ | 16=¹/₁₉ |
| Buccal cavity | 22=¹/₁₅ | 19=¹/₁₅ | 13=¹/₇ |
| Spicules | | 72 | |

The size of adult females varies between 2,300 and 3,000 □; that of males between 1,350 and 1,970 □.

The body of the females (pl. XVII, fig. 4), of almost cylindrical shape, becomes slightly and gradually thinner in the front, where it

is terminated by the truncation of the mouth. At the back (pl. XVII, fig. 7), it ends with an oblong ovoid shape, extended by a narrow tail that thins out suddenly. Observed with transmitted light, it takes the opaque blackish aspect known in *Rhabditis*. This opacity is caused by the many granules which the cells of the intestine are loaded with. The male is shorter and also always proportionally thinner and more slender.

The cuticle, colorless and rather transparent, is smooth, without striation nor ornament of any kind. I have not verified the existence of a lateral membrane.

The mouth ends the anterior extremity (pl. XVII, fig. 5) without any special narrowing that would distinguish it from the rest of the body. It is lined with three large serrated lips, that bear a thin papilla on each of their lobes, six in total. The cuticle, very thin on these lips, makes them appear as if protruding from a sheath. – The buccal cavity, of 18 to 20 μ in length, has the shape of a regular, strongly chitinized cylinder with a relatively well-developed posterior narrowing.

The esophagus (pl. XVII, fig. 6), from 280 to 340 μ in length in the females and from 230 to 280 μ in the males, has the shape that is usual in *Rhabditis*, with its two clearly bulging bulbs. The posterior bulb, slightly pear-shaped, is provided with very little developed valves (teeth). The esophagus sheath comes upwards almost until the anterior extremity of the buccal cavity (pl. XVII, fig. 5).

The tail of females (pl. XVII, fig. 7), 105 to 145 μ in length, starts from the anus with an ovoid shape in its first anterior third, then suddenly narrows like a slender awl with a sharp tip, bent slightly sideways. Exactly at the level of this narrowing are found two thin

lateral papillae. This conformation of the tail is characteristic for the species and allows it to be easily distinguished.

The cells of the intestine of well-fed animals contain many granules that make it appear with an opaque blackish aspect. In the posterior third of the intestine, these granules are in great part made up of birefringent corpuscles, whereas in the two anterior thirds, these corpuscles are not found and all granules are of albumino-fatty nature.

I did not study the cellular structure of the intestine, but I believe it to be identical to that of the intestine of *Rhabditis elegans*, that is, made of two rows of few large cells. The young larvae after hatching (pl. XVIII, fig. 2) are in this respect absolutely identical to that of *R. elegans*, with the same number of nuclei and the same arrangement.

The nerve ring (pl. XVII, fig. 6, c) appears with the usual shape and arrangement. It embraces the narrow portion of the esophagus in between the two bulbs, bending over obliquely towards the ventral side.

The excretory apparatus (pl. XVII, fig. 6, c) opens through a thin chitinized canal into the pore located at the level of the posterior bulb.

The female genital apparatus (pl. XVII, fig. 4) has a general conformation that is absolutely identical to that of *Rhabditis elegans*. It differs from it by the greater dimension of the uteri, which may each contain as many as 50 to 60 eggs. It also differs from it by the absence of a rachis in the folded portion of the ovary that constitutes the germigen. This folded portion never extends until the level of the vulva. The tuba, or oviduct, also serves as a seminal receptacle. – The eggs, of a cylindrical rather than oval

shape, measure 60 \square in length and 37 \square in width. Their shell is smooth.

The tail of the male (pl. XVIII, fig. 1, A, B) widens in a wide bursa that is open in the front and of the peloderan type, that is completely enveloping the extremity of the tail. This extremity merges without definite boundary with the narrowing posterior part of the bursa. The latter is relatively long, and extends forwards until the level of the anterior end of the spicules. It is equipped with 9 pairs of papillae, arranged in groups of three: a rather tight terminal posterior group, a less tight median group, finally an anterior group with very distant papillae. Only these three latter papillae are preanal. Finally an unpaired papilla is also found on the anterior rim of the anus.

The spicules and the gubernaculum, or accessory piece, are of a dark brown color. These pieces (pl. XVIII, fig. 1, A, B) are very long and very vigorous. The spicules measure from 70 to 85 \square . They are independent of each other. Their tip is thick and blunt and they widen up slightly in the front. The gubernaculum measures approximately a third of the length of the spicules.

Nucleated rectal glands are found on the dorsal and ventral sides of the junction point between the intestine and the vas deferens.

The testis is built according to the usual shape in Rhabditis. On all observed individuals, I always saw the seminal reservoir full of spermatozoa. These spermatozoa (pl. XVII, fig. 11), of identical shapes and dimensions in males and females, are quite voluminous. Of spherical shape, they measure 9 \square in diameter. Their plasma is finely granular and, in the center, it is easy to distinguish a small compact nucleus. I have never seen them execute the least movement.

Rhabditis Caussaneli is ovo-viviparous, but mostly oviparous. The eggs are stored in the uteri, where they usually remain until the time of hatching. They are thus laid at a stage of development that is almost always very advanced. In the fully adult females, fifty to sixty eggs can be counted in each uterus. Some of these eggs hatch in the uterus, and the juveniles are expelled outside together with the yet unhatched eggs. Towards the end of the production of fertilized eggs, these intra-uterine hatchings increase in number. The juveniles then find their food in the uterus at the expense of the unfertilized shell-less eggs which start to arrive. They grow in this enclosed environment and finally tear apart the walls by wriggling about. They then spread into the general body cavity and attack the viscera of their mother. The latter perishes being devoured from the interior by its children. Only the cuticle remains, forming a long transparent bag, in which the young *Rhabditis* can be seen to move about. At least half of the females end thus. The others continue to produce shell-less sterile eggs, which fall apart immediately when laid. These sterile eggs in addition accumulate inside the uteri, which are often crammed and bloated with them. This production of sterile eggs may continue for five or six days, before these females die of exhaustion and old age.

I have isolated females on several occasions, so as to have an idea of the number of fertilized eggs that they could lay. The figures varied from 250 to 300, whence one must conclude that the female genital gland, in its functioning as a testis, is limited to a production of 250 to 300 spermatozoa. These 250 to 300 fertilized eggs, at a temperature of 20° C., are laid within three days. At the same temperature, these females may still lay for another 6 days, after exhaustion of their stock of sperm. It must be concluded that their

genital gland is able to produce three times more ova than spermatozoa and that two thirds of this production are completely useless and fated in advance to destruction.

The existence of this nematode is as short and fast as that of *Rhabditis elegans*. From the details in which we entered above regarding the layings, it can be seen that this existence flows and ends in an identical fashion to that of its congener.

The juveniles that have just hatched (pl. XVIII, fig. 2) measure approximately 300 μ . They already possess all their complete organs, except the still very rudimentary genital organ. As in *Rhabditis elegans*, the proportions of the body parts are very different from those in the adult state. The esophagus equals $1/3$ and the tail $1/9$ of the total length instead of $1/9$ and $1/24$. It results thereof that, in the final growth, the largest part is provided by the middle region, which expands 6 to 7-fold, whereas the esophagus and the tail only expand 3 to 4-fold. – The intestine is made of 18 cells, arranged in two rows and indicated by 18 voluminous nuclei endowed with a large nucleolus (pl. XVII, fig. 9). – The genital primordium (pl. XVII, fig. 10) is made up of two large nucleolated germinal nuclei in the center and two small somatic cells, one at each end, the whole being enveloped by a thin anhistic membrane.

The molt, the encystment and the growth of this species have been studied at length in my work on molt and encystment, pages 578 and 606¹.

Rhabditis Caussaneli is quite agile. It moves using undulatory movements; but only with difficulty does it succeed to come out of the water drops in which it is cultured. Its culture on a depression slide is very easy, by feeding it with rotten flesh.

¹ Archives de Zoologie, t. VII, 1899.

One could be willing to relate this species to the *Rhabditis (Leptodera) fœcunda* of Schneider, also found by the German scientist in Arions¹. Unfortunately, we know the latter only through two very rough sketches of the esophagus and of the female tail. However, the configuration and the proportions of the latter are so different from those of *R. Caussaneli* that it appears to me impossible to liken the two forms. With the characteristics of the male that I could describe, the latter constitutes a well-defined species.

Once I had well recognized the protandrous autogamous hermaphroditic state of this species, I thought to search whether I could find males, as in *Rhabditis elegans*. I proceeded in these researches in an identical fashion to that described above concerning the latter.

In the month of October and of November, 14 cultures were thus organized, sorted and counted. I found 14,740 individuals, among which 14,719 females and 21 males: that is 1.4 ♂ for 1,000 ♀. The proportion is identical to that found by us in *Rhabditis elegans*. Also as in the latter, this proportion is an average drawn from all my cultures, but not rigorously exact for each of them. In fact, in two of them, containing for the first 876 and for the second 1,160 individuals, I found 3 and 6 males, respectively, that is 3.4 ♂ for 1,000 females; whereas in four others, adding up to a total of 3,742 individuals, I did not encounter a single one.

These 21 males appeared to me of sound constitution. Shorter and thinner than the females, they moved about rapidly among them in a perpetual movement and agitation. The bursa, the spicules and the testis showed a normal organization. The seminal reservoir was

¹ *Monographie der Nematoden*, p. 315, pl. X, fig. 11, a, b.

crammed with spermatozoa, of identical volume, structure and conformation to those produced by the genital gland of hermaphroditic females. On all these matters, they behaved in a perfectly similar fashion to those of *Rhabditis elegans*.

After having recognized the existence of these males, I undertook some experiments to see whether it would be possible to determine the conditions of their appearances and to increase their number at will.

I first tried the influence of insufficient nutrition. So that one can see the conditions under which I operated, I reproduce in full the journal of this experiment.

(11 January, 16° C.) Isolated, in a drop of pure water, 8 larvae undergoing their second molt and still dragging along their molt cast behind them.

(12 – 16°) Although fully deprived of food, these third-stage larvae continued to develop and, at 10 o'clock in the morning, some of them started to undergo their third molt.

(13 – 16°) 12 a. m. They are very emaciated. The body has become transparent and, in the polarized light, I do not see any hint of birefringent corpuscles. The cells of the intestine are vitreous. Added some rotten flesh. – 7 p. m. The animals which were well fed this afternoon have recovered all their vigor. The cells of the intestine are full of albumino-fatty granules, which make the intestine blackish and opaque along all its length, a sign of plentiful food. I place them again in a drop of pure water.

(14 – 16°) 6 a. m. The larvae have fasted all night and appear rather emaciated. Given new food. – 11 a. m. Well fed; the cells of the intestine have been replenished with granules and the intestine has an opaque blackish aspect. Placed again in a drop of pure water.

(15 – 16°) 5 a. m. The animals have undergone their fourth molt. They are very emaciated, the intestinal cells clear and devoid of granules. Given food again. – 12 a. m. Ate well: opaque blackish intestine: replaced in pure water. – 8 p. m. Very emaciated: given food again.

(16 – 16°) 6 a. m. Intestine again blackish and full of granules: placed again in pure water. – 8 a. m. Start to lay. – 8 p. m. Rather emaciated: 24 eggs: given food again.

(17 – 15°) 8 a. m. The animals fed all night have laid a fair number of eggs: the intestine appears again blackish opaque because of the accumulation of granules. Although these animals are fully adults, they do not reach the normal length and the diameter of the middle of the body is relatively small. Indeed, it is only of 70 to 80 μ a figure well below that of normally reared individuals, for which it rises to 140 μ . This reduced diameter is obviously the result of a poor development of the genital organs. Placed again in pure water. – 8 p. m. Emaciated; little laid; given food again.

(18 – 14°) 10 a. m. Intestine again opaque blackish; replaced in pure water. – 9 p. m. Laid few eggs: given food again.

(19 – 14°) 8 a. m. Laid a fair number of eggs: placed again in pure water. – 8 p. m. Continued to lay; emaciated; given food again.

(20 – 14°) 6 a. m. Laid: a dead mother; placed again the others in pure water. – 8 p. m. Given food again.

(21 – 15°) Laid again some good eggs during the night; but the seven females have exhausted their stock of spermatozoa and henceforth only produce unfertilized eggs, which fall apart immediately.

Listing of the individuals that became adults from each of the layings:

| | | | ♀ | ♂ |
|---------------|-------------------|-----------|---------|---|
| (22 — 15°) | Layings of 16, 17 | 110 | 0 | |
| (23 — 15°) | — | 18 | 213 | 0 |
| (24 — 14°) | — | 19 | 195 | 0 |
| (25 — 13°) | — | 20 | 124 | 1 |
| (26 — 12°) | — | 21 | 76 | 0 |
| Totals. . . . | | <hr/> 718 | <hr/> 1 | |

Thus, during the course of this experiment, the larvae were totally deprived of food during the entire length of the third stage and of the third molt. This stage is quite important for the development and the growth of genital organs. During the fourth stage, far more important, they were subjected to three cycles of abundance and starvation and finally underwent their fourth molt in dearth. During the fifth stage, which is the period of maturation of ova, these cycles of abundance and starvation were repeated six times.

Nonetheless, these frequent and complete changes in food conditions do not seem to have had the least effect on the sexuality of the products of these eight females. They only considerably reduced their number, since on average, each of them laid only ninety fertilized eggs, whereas for correctly fed females, this average approaches 300; this is a reduction of two thirds of the normal number of layings. We conclude therefore, as we previously did concerning *Rhabditis elegans*, that the dearth of food has no determining influence on the sexuality of this nematode.

I also tried to obtain heterogamous fertilizations, either with young females that had not yet begun to lay, or with old females that had exhausted their own stock of sperm. I proceeded in an

absolutely identical manner to that described above for *Rhabditis elegans*.

In these nine experiments, twenty-five males were isolated with thirty-two females that had exhausted their stock of sperm, and ten males with ten young females that had not yet begun to lay, total: thirty-five males and forty-two females. I observed with the greatest care and very frequently the assembled couples, which lived in this manner for times varying from five to ten days. Never did I see a male make the least attempt to approach a female and, *a fortiori*, never could I witness a mating. Thus the thirty-two females, having exhausted their stock of sperm, finished their existence by only laying unfertilized eggs. The ten young females first laid the quantity of fertilized eggs that is characteristic of these hermaphrodites, and from these eggs, only females came out. The ten mothers then, like the thirty-two others, ended their existence laying sterile eggs.

One must therefore conclude that, in this species, the males have become unable to mate, despite their apparently perfect organization. Indeed, this inability does not appear to derive from a defect in the organization of the testis. This organ was very normally constituted in all observed males and the seminal reservoir always packed with well-conformed spermatozoa. The copulatory organs (bursa, spicules) had a very good appearance. By their whole morphology, these males had a perfect exterior. That being the case, we must admit that only the sexual instinct, the reproductive sense, is abolished in them.

In order to verify whether the hermaphroditic generations could maintain and reproduce themselves indefinitely through simple autogamous fertilization, I undertook similar cultures to those

described above for *Rhabditis elegans*. The processes and methods were absolutely identical.

A first culture, started on the 1st of October, lasted until June 9, date at which it got extinguished at the 49th generation. Until then, I had not noticed any sign of degeneration, and the previous generations appeared as vigorous as the beginning of the culture.

For reasons that are not useful to expose here, the 48th generation was fed rather badly during its larval period. When the larvae reached the adult age, they were better provided and I kept a hundred of them as reproducers. The temperature had risen to 23 or 24° C. I noticed then that, in many of these adult females, the ill-developed genital organs were not producing any eggs nor spermatozoa. In others, these organs, probably less atrophied, gave a few eggs, which remained however sterile because of the lack of spermatozoa to fertilize them. In some rare individuals only, the production of eggs was preceded by the formation of a few spermatozoa, and, by gathering them all with the greatest care, I succeeded in collecting about thirty fertilized eggs, from which as many larvae came out, which formed the 49th generation.

These laying females, so ill-fitted from the genital standpoint, were very well conformed from the vegetative standpoint. Regarding their size and the organization of their intestine, they did not differ at all from the laying females of the previous generations. They actually did live as long. Their degeneration had thus only concerned the genital apparatus.

The thirty larvae of the 49th generation were raised with the greatest care, but a good half remained atrophied and did not reach the normal adult state. Among those that reached it, some were completely sterile and the others laid some unfertilized eggs, as in

the previous generation. Thus, not a single one of them had produced spermatozoa. From the vegetative standpoint, these latter laying females were well conformed and lived a normal life.

A second culture that was begun in the month of November became also extinct in the following June after having reached the 41st generation. It had behaved normally until the 39th generation. The generations followed each other regularly, the animals being strong and vigorous, laying the normal number of fertilized eggs that hatched in good conditions.

But with the 39th generation appeared degeneration phenomena that were very similar to those described above in the culture of the previous year, exactly at the same time. From this 39th generation, I only managed to rear hundred fifty individuals, which formed the 40th generation. The latter turned out to be even more unfertile than the 39th, since keeping all the layings, I could only bring eighty individuals to the adult state. The latter, representing the whole 41st generation, were all absolutely sterile. I kept them by feeding them well until their natural death; not a single one laid any eggs.

It seems to me that this sterility, thus reappearing in two following years at the same period, and showing itself under identical forms, can only be attributed to temperature. The latter, in both cases, had just reached 22 to 23° C. This is actually the conclusion to which we were brought by the cultures of *Rhabditis elegans* described above.

RHABDITIS MARIONIS mihi

I found this species on rotten earthworms (*Lumbricus*) that had been sent to me from Vire, in Normandy. There were several putrefying worms. Only one of them swarmed with nematodes, which made me think that the latter derived from a few encysted

larvae, for which the infested worm, when it was alive, had served as a host. Through the absence of males, I soon realized that I was dealing with an autogamous protandrous hermaphroditic species. I established a culture of it in depression slides, where I held it in observation for five to six months. I dedicate it to the memory of Mr. Marion, professor at the Faculty of Sciences in Marseilles, whose recent death French zoology deplores.

Measurements:

| | ♀ | ♂ | YOUNG at hatching |
|---------------------|----------|---------|----------------------|
| Body | 2030 □ | 1430 □ | 325 □ |
| Esophagus | 257=¹/₈ | 228=¹/₆ | 100=¹/₃ |
| Tail | 128=¹/₁₆ | 56=¹/₂₆ | 70=¹/₄.₆ |
| Vulva | 1029 | | |
| Diameter | 100=¹/₂₀ | 56=¹/₂₆ | 17=¹/₁₈ |
| Buccal cavity | 28=¹/₉ | 26=¹/₉ | 16=¹/₆ |
| Spicules | | 60 | |

The size of the females varies between 1850 and 2050 □: that of the males between 1300 and 1450 □.

The body of the females (pl. XVIII, fig. 3) is spindle-shaped, very long, and narrows out slowly towards both extremities. Whereas it is truncated in the front, it ends by a thin tip in the rear. When observed under transmitted light, it takes an opaque blackish aspect, which is caused by the many granules that are deposited in the cells of the intestine. – As in the other species, the body of the males is always shorter and more slender, and approximately cylindrical along its whole length.

The cuticle is thin, colorless and transparent. It bears a thin transversal striation, so delicate that I very often happened to fully question its existence on some specimens.

The mouth (pl. XVIII, fig. 4) is lined with three little protruding lips, each divided into two lobes by a shallow serration. Each lobe bears a thin papilla. – The buccal cavity, of 28 μ in length, is perfectly cylindrical; its chitinized walls are thick. The narrowing and the terminal thickening that connects it to the esophagus are well developed.

The esophagus (pl. XVIII, fig. 5) presents the usual overall conformation. Its two bulbs, of rounded shape, are well developed. The valves (teeth) of the posterior one are vigorous. The esophageal sheath of the buccal cavity envelops upwards the posterior three quarters of the latter.

The female tail (pl. XVIII, fig. 6), which measures 115 to 130 μ , narrows at first quite rapidly, then thins out gradually to end in an extremely fine tip. It bears a pair of thin lateral papillae, which are inserted slightly beyond the end of its anterior third. An internal extension of these papillae, in the shape of a thin fiber, can be followed until the level of the anus.

The intestine appears opaque blackish under transmitted light. This opaqueness is caused by the many granules with which its cells are loaded. Some of these granules are of proteinic and fatty nature, the others birefringent crystalline. The latter accumulate particularly in the last posterior quarter of the intestine. In well-fed individuals, the cells of this region are literally crammed with these birefringent corpuscles, whereas they are far less abundant in the other three quarters of the intestine. In ill-fed individuals, they are only found in the cells of the last quarter. These birefringent corpuscles are of spherical shape and rarely exceed a diameter of 2.5 μ . – The encysted larvae, literally crammed with albumino-fatty granules, do not display any birefringent corpuscles.

The rectum bears at its origin a pair of nucleated glands (pl. XVIII, fig. 6, *g*).

I have not pursued any researches on the cellular structure of the intestine; but I have good reasons to believe that it has an identical composition to that of *Rhabditis elegans*.

The nerve ring (pl. XVIII, fig. 5, *c*), of fibrous structure, envelops the esophagus at the narrowing between the two bulbs. It bends obliquely towards the ventral side and sends out extensions directed towards the excretory pore.

The excretory apparatus (pl. XVIII, fig. 5, *c*) is somewhat difficult to see. The unpaired canal that leads to the pore is thin and short. I succeeded in following the anterior and posterior lateral branches on a rather long stretch. A unicellular gland with a large nucleolated nucleus is found closely behind the pore.

The vulva (pl. XVIII, fig. 3, *v*), located quite exactly at the middle of the body length, has lips that hardly protrude. The vagina is very short. The uteri are short and do not ever contain more than four to five eggs. The oviduct (tuba), compressed in the shape of an S, also serves as a seminal receptacle. The vitellogen is relatively long and voluminous and contains a certain number of growing ova. The germigen, folded in the reverse direction, extends until the level of the vulva. The anterior and posterior bends of the genital organ are always quite far from the esophagus on one side, from the origin of the rectum on the other side. – The eggs (pl. XVIII, fig. 9), of oblong shape, measure 72 \square in length and 33 \square in width.

The bursa of the male tail (pl. XVIII, fig. 7, A and B), open in the front, is wide and well developed. It belongs to the leptoderan type, that is to say that it lets through a free caudal extension at its posterior end. It is serrated at the point of emergence. The free

caudal tip is thin, awl-shaped and rather short. The 9 papillae are arranged in groups of three: 1^o a rather tight posterior group; 2^o an equally tight median group; 3^o a preanal group with first two neighboring papillae, then the last one, which is rather far apart in the front. Ventral and dorsal anal glands (pl. XVIII, fig. 7, B) are found at the junction point between the intestine and the vas deferens.

The spicules (pl. XVIII, fig. 7 and 8), of 55 to 60 μ in length, are colorless and vigorous. They end into a rather fine tip and thicken strongly towards the front. The gubernaculum (accessory piece), seen from the side, has the shape of a rod. It is relatively long, its length slightly exceeding half of that of the spicules. The latter are completely free and independent of each other.

The testis, built according to the usual type, does not offer anything noteworthy. The spermatozoa, whether from the females or from the males, are absolutely identical in their shape, structure and volume. Of spherical and slightly oval shape (pl. XVIII, fig. 10), they measure 8 μ . Their finely granular cytoplasm envelops a very apparent small nucleus of a somewhat irregular shape. I never observed the least sign of motility of these spermatozoa.

Rhabditis Marionis is essentially oviparous. It lays its eggs as they mature, often before their first cleavage, at the latest after the third or the fourth. Therefore, never more than five to six eggs can be seen at any time within each uterus.

In order to have an idea of the number of fertilized eggs that this species could lay, I isolated a female, which I am going to tell the whole story of, because of the important fact that she gave me the opportunity to witness for the first time.

This female was obtained from an egg that was isolated as it was just laid. The temperature during the course of its culture was of 20° C. Hatching occurred 20 hours after the laying. The well-fed young larva took three days and a half to grow until the laying of its first egg. Then, on the first day it laid 61 fertilized eggs, on the second 48 and on the third 20, total 129; afterwards it only laid sterile eggs that fell apart rapidly. It had completely exhausted its stock of sperm.

These numbers of 61, 48, 20 and this total of 129 were very low, compared to those that I had obtained in the other hermaphrodites. The explanation came to me with the following observation. Already on the first day, by observing the mother when she had just laid its three to four first fertile eggs, I noticed besides these the presence of an equal number of shell-less eggs that were falling apart, which had obviously not been fertilized. Moreover, on the next day, I found about thirty of these unfertilized eggs next to the 48 that were developing regularly. From then on, there was no possible doubt: I had a female of which one of the ovaries had functioned according to the hermaphroditic mode, first producing spermatozoa, then ova, whereas her other ovary had functioned according to the dioicious mode, only producing ova. We shall see below that this case is rather frequent in this species.

By doubling the number of fertilized eggs, an average figure of 250 to 260 is reached, which quite precisely corresponds to those obtained in the previous species.

After having laid her 20 last fertilized eggs, the mother still lived for 24 days, laying unfertilized eggs almost until the end. If the incubation period of the egg is taken into account, this female had lived for 31 days. This length is probably one of the longest that a *Rhabditis Marionis* can attain. For in another culture where I had

gathered about forty females, I saw them all becoming extinguished after 12 to 16 days of existence, the temperature being, of course, the same.

The juveniles that have just hatched (pl. XVIII, fig. 11) measure 320 to 330 μ . As in the previous species, they already possess all their organs, except for the genital apparatus, still in a very rudimentary state. The proportions of the different parts of body are however very different from those in the adult state; the esophagus measuring $1/3$ and the tail $1/4$ of the total length, instead of $1/8$ and $1/16$. It follows that during the final growth, the greatest part of it concerns the intestinal median region, which expands 12-fold, whereas the esophagus and the tail only expand 2 to 3-fold.

I have not observed the molts of this species; its encystment has been described in my work on molt and encystment¹.

This species is a little slow and heavy. It moves little about in the water drops where it is held in culture. Its culture is easy on a depression slide, feeding it rotten flesh.

Among the other previously described *Rhabditis*, the only one with which one could be tempted to confuse it would be the *R. Verneti (terricola)* of Vernet. But it can be distinguished by the rounded shape of the esophagus bulbs, by the more posterior position of the excretory pore, by its longer buccal cavity, by its shorter and tapered tail, finally by its oviparity.

Once I had noticed the existence of males in *Rhabditis marioni*, I organized cultures in order to know their frequency. These cultures were arranged and studied with the same methods as described for *R. elegans*. Eight cultures thus sorted and numbered gave me a total of 2,100 females and 93 males, thus 7.6 males for 1,000 females.

¹ *Archives de Zoologie*, t. VII, 1899, pl. XVIII, fig. 26.

This proportion is an average figure for the whole set; but in some of the cultures it rose to 13 for 1,000 and in others to only 2 for 1,000. In any case, this proportion is three to four times larger than in the two previous species.

These males, like those of the two already described species, were of very good constitution in all of their parts. Nimble and agile, they circulated rapidly in the middle of the swarming of the females. Their seminal reservoir was full of spermatozoa that were absolutely identical to those of the females.

On these cultures, I also ascertained the frequency in this species of the partial and incomplete hermaphroditism mentioned above regarding the female that was isolated and reared apart. Indeed, in five of these cultures, I found from the first day on a rather large number of unfertilized eggs. Since all the animals in these abundantly provided cultures were very vigorous, these layings of sterile eggs could not be attributed to a withering state of some individuals. A partial hermaphroditism of some females must therefore be admitted, as we have demonstrated above for the isolated female. I even observed one in which both genital glands had come back to be producers of only ova. These facts are very interesting. They prove us that, in *Rhabditis Marionis*, hermaphroditism is not yet fixed in an absolute fashion and that it can totally disappear under the influence of unknown causes. We will actually have to return more than once to this point, regarding species in which we again will find this incomplete hermaphroditism under a more marked form.

As with the two previous species, I have tried to obtain heterogamous refertilizations, by gathering and isolating together males and females that had exhausted their own stock of sperm. Five

experiments were thus instituted with a total of 28 females and 42 males. A single one, composed of 5 females and 2 males, gave an entirely negative result, none of the females having been refertilized, even though they had lived for seven days with their males. On the four other preparations, on the contrary, a great part, the majority I believe, of the females were refertilized and laid numerous well-constituted eggs during the five to six days that they spent in the company of males. The number of unfertilized eggs, the presence of which was ascertained every day, indicated however that some of the females were never visited by the males, even though the latter were in almost two-fold number. In any case, the higher proportion of refertilizations proves that the reproductive sense of these males was less atrophied than in the previous species. This agrees with the also higher proportional number of males, and these two facts, with the frequency of monoic females, tend to clearly demonstrate that the hermaphroditism of *Rhabditis Marionis* is less developed and less complete than that of *R. elegans* and *Caussaneli*.

Juveniles that came from 150 to 200 eggs originating from heterogamous refertilization were kept and raised until the adult state. All became protandrous hermaphroditic females, which started to lay regularly developing eggs. The heterogamous fertilization thus had, in this *Rhabditis*, no arrhenotokous influence such as that ascertained in *Rhabditis elegans*.

RHABDITIS DUTHIERSI mihi

I found this species once, in a blackish soil sampled in the forests of the Edough, next to Bône. I brought it into culture on depression slides and let it multiply by feeding it rotten flesh. I dedicate it to my

illustrious teacher, Mr. de Lacaze-Duthiers, professor at the Sorbonne.

Measurements:

| | ♀ | ♂ |
|---------------------|----------|-----------|
| Body | 1830 □ | 1430 □ |
| Esophagus | 243=¹/₇ | 214=¹/₆.₆ |
| Tail | 255=¹/₈ | 71=¹/₂₀ |
| Vulva | 858 | |
| Diameter | 100=¹/₁₈ | 64=¹/₂₂ |
| Buccal cavity | 28=¹/₉ | 28=¹/₈ |
| Spicules | | 45 |

The body of the females (pl. XIX, fig. 1), cylindrical in its median part, narrows at both extremities. In the front, it is truncated by the mouth; in the rear, it thins out in a fine tip. The body of the males, as usual, is always shorter and thinner.

The cuticle is colorless, thick, highly birefringent and bears fine transverse striations. These striations, on living material, require a high magnification to be seen; but after incubation in 1 % acetic acid they become easily visible. I did not see lateral membranes. The cuticle is composed of two layers, which clearly separate under the action of 1 % acetic acid.

The mouth (pl. XIX, fig. 2) is not differentiated from the rest of the body by any narrowing nor protuberance of any sort. It is lined with three scarcely protruding lips, slightly serrated into two little-distinct lobes, each bearing a papilla; the latter thus numbering six around the full circumference. The buccal cavity has the shape of a perfect cylinder with thick walls. The posterior constriction and bulge are clearly outlined.

The esophagus (pl. XIX, fig. 3), modeled according to the usual type, is remarkable by the exceptional bulge of its two bulbs. The valves (teeth) of the posterior bulb are well developed and vigorous.

In the front, the esophagus sheath envelops the buccal cavity, reaching upwards slightly beyond the posterior extremity of the first third of its length.

The tail of the females (pl. XIX, fig. 4) thins out rapidly and ends in a long and very thin tip. It is slightly flexuous and undulating. It bears a pair of fine lateral papillae (pl. XIX, fig. 4, *p*), inserted behind the anus at a distance slightly shorter than the length of the rectum.

The intestine, seen with transmitted light, has an opaque blackish aspect, caused by the many granulations stored in these cells. The length of the rectum equals the diameter of the body at the level of its anterior end. It is endowed, at this end, with dorsal and ventral glands, within which a large nucleolated nucleus can be seen.

The nerve ring (pl. XIX, fig. 3, *c*), of fibrous structure, collars the esophagus at the constricted neck between the two bulbs, bending over obliquely towards the ventral side and sending extensions into the direction of the excretory pore. The latter is located at the level (pl. XIX, fig. 3, *p*) of the posterior bulb. The unpaired canal is strongly chitinized. I have no observations concerning the lateral branches.

The vulva (pl. XIX, fig. 1, *v*), located slightly anteriorly to the middle of the whole body length, bears scarcely protruding lips. The vagina is very short. The paired genital tube is little developed. Its anterior and posterior bends terminate far away, on one side at the back of the esophagus, on the other side in front of the rectum. The very short uteri do not ever contain more than five or six eggs each. The oviduct or tuba also serves as a seminal receptacle. The germigen, which extends until the bend, contains 7 to 8 ovules. The

ovary, folded in the other direction, always ends slightly anteriorly to the level of the vulva.

The eggs (pl. XIX, fig. 5), of oblong shape and rounded at the extremities, measure 60 \square in length and 33 in width.

The male tail (pl. XIX, fig. 6, A, B) opens up in a wide bursa, opened in the front and with an elliptical contour at the back. It belongs to the leptoderan type, that is with a free caudal extension at the back. This extension has the shape of a thin awl-shaped tip of a length equal to approximately half that of the spicules. The nine papillae are arranged in four groups: 1^o a rather tight posterior group; 2^o a median group, also of three, which are less tight; 3^o a group of two that are not very close; 4^o finally a last isolated single papilla, far apart anteriorly, at the level of the anterior extremity of the spicules. Only the last three papillae are preanal.

The spicules (pl. XIX, fig. 6 and 7), of a slightly smoky color, are very strong, very thick and quite arched. They end with a rounded thick tip, bilobed, very characteristic for the species. The gubernaculum (accessory piece) is also vigorous and thick; its length almost equals half of that of the spicules. The latter are free and independent of each other.

The testis, built according to the ordinary type, does not offer anything peculiarly noteworthy. In all observed males, it appeared to me well constituted and the seminal reservoir was full of spermatozoa. These (pl. XVIII, fig. 12) are absolutely identical to each other, whether of masculine or of feminine origin. Of spherical shape, they are quite voluminous and measure 12 to 14 \square in diameter. Their finely granular cytoplasm envelops a small opaque nucleus, of a slightly irregular shape. When treated with acetic acid, this nucleus appeared to me composed of five small spherical

chromosomes (pl. XVIII, fig. 13). I did not observe any sign of motility in the spermatozoa.

Rhabditis Duthiersi is oviparous. Its little developed uteri never contain more than 5 to 6 eggs and these are always laid at a rather early stage of development. It lays 200 to 210 fertilized eggs, after what, its stock of sperm being exhausted, it only lays unfertilized eggs. These sterile layings can last for 6 to 8 days and thus emit two to three times more sterile eggs than fertilized eggs. At a temperature of 20° C., it lays a daily average of 100 eggs.

The existence of this nematode is slightly slower and longer than that of *Rhabditis elegans* and *R. Caussaneli*. At a temperature of 20° C., its eggs take 24 to 26 hours to develop until hatching. The juveniles then grow for three days until the laying of the first egg. The fertile laying lasts two days to two and half days. Thereafter, the sterile layings can last for six to eight days, still followed by three to four days of absolute sterility, leading to death. The total length is thus of twenty to twenty-three days.

The movements of *Rhabditis Duthiersi* are slow and heavy. It wriggles and moves very little.

This *Rhabditis* is close to *Rhabditis elegans* and *R. Marionis*. It can be distinguished from the former by its longer esophagus and tail, by its cylindrical buccal cavity, by its oviparity and above all by its leptoderan bursa, with longer and stronger spicules. The similarity with the latter is closer. It can be however easily distinguished by the much longer female tail, by the male caudal papillae, the last of which is moved further forwards, and above all by its spicules, shorter but more thickset and ending in a round bilobed tip.

As for the previous species, I wanted to ascertain the frequency of males using cultures that were organized and studied with the method described for *Rhabditis elegans*. These four cultures gave me a total of 1,279 females and 37 males; thus 20 males for 1,000 females. This proportion is an average drawn from the total; but in one of the cultures, it rose to 35 for 1,000, whereas in another, composed of 461 individuals, I did not find a single male. The general proportion is much greater than those found in the previous species.

I observed three females, which, from the beginning of their layings, simultaneously laid fertilized eggs that developed normally, and sterile shell-less eggs that were falling apart. They continued in this manner for three days, then only laid sterile eggs. It is obvious that these three females had formed spermatozoa in one of their genital glands only and that I had there cases of partial or incomplete hermaphroditism, similar to those that we already observed in *Rhabditis Marionis*.

I tried to obtain in *Rhabditis Duthiersi* heterogamous fertilizations with females that had exhausted their own stock of sperm. Three series were organized, in which 62 females and 41 males were held together during 3, 4 and 6 days, respectively. Only in the first series did a useful and fertilizing mating occur. Indeed, for three days I found in this preparation layings of fertilized eggs that hatched and gave birth to regularly developing juveniles. When at the adult stage, these juveniles gave 70 females and 1 male. It must be concluded that the fertilization through heterogamy did not have any determining action on the sexuality of its products, in contrast to what we had observed in *Rhabditis elegans*.

In *Rhabditis Duthiersi*, as for all already studied dioico-hermaphroditic species, the males seem to have lost, in great part if not totally, their sexual instinct. They can be seen circulating with indifference among the females, bumping and rubbing them, as they would do with any inert bodies. Mating attempts can be observed only very rarely. However, they appear to be well constituted males. Their testis produces plenty of beautiful spermatozoa, which fill the seminal reservoir. Only their reproductive sense seems to be completely lacking.

RHABDITIS PERRIERI mihi

I found this species only once, in soil sampled at the bottom of a manure heap in the plain of Mitidja, in the Arba. I raised and cultured it on depression slides. I dedicate it to Mr. Ed. Perrier, the learned director of the Museum.

Measurements:

| | ♀ | ♂ |
|---------------------|---------|---------|
| Body | 1358 ♂ | 929 ♂ |
| Esophagus | 185=¹/₇ | 171=¹/₅ |
| Tail | 171=¹/₈ | 42=¹/₂₂ |
| Vulva | 715 | |
| Diameter | 57=¹/₂₄ | 38=¹/₂₄ |
| Buccal cavity | 19=¹/₁₀ | 18=¹/₉ |
| Spicules | | 32 |

The size of adult females is quite uniform and oscillates between 1300 and 1400 ♂. The body (pl. XIX, fig. 8), cylindrical in its median region, tapers gradually towards both ends. In the front, it ends by the truncation of the mouth; in the rear, it thins out into a long tail, which is always slightly curved on the dorsal side. With transmitted light, it has a general dark greyish aspect, never blackish as in most

Rhabditis. We shall see later, when speaking about the intestine, the cause for this difference. The male is always shorter and thinner.

The cuticle is colorless, smooth, without striation nor ornament of any sort. The lateral membrane protrudes very little and presents the aspect of a narrow band, marked with four to five longitudinal lines.

The lateral bands are wide and granular; I did not see any nuclei. The dorso-ventral bands are very narrow. The wide muscular bands belong to the meromyarian type. They are composed of large spindle-shaped cells, longitudinally striated as a result of their fibrillar structure. I did not see any nuclei in these cells.

The mouth (pl. XIX, fig. 9) ends the anterior extremity without special differentiation that would distinguish it from the rest of the body. In its whole, it has the slightly rounded shape of a dome. It is lined by six small lips, that are so difficult to distinguish that I hesitated for a long time about their number, often believing that I was seeing only four of them. Each of these lips appeared to me to be equipped with an extremely fine papilla. – The buccal cavity, with a length of 19 \square , is regularly cylindrical and with thick walls. At its posterior end, the walls become thinner until they connect to the usual terminal thickening, without any intermediate constriction.

The esophagus (pl. XIX, fig. 10) presents the usual conformation. The two bulbs have a rounded spherical shape. The constricted intercalary neck is relatively quite long. The valves (teeth) of the posterior bulb are strong and vigorous. One or two nuclei can be always distinguished within the fleshy mass of this bulb. In the front, the esophagus sheath comes upwards until the anterior third of the buccal cavity.

The female tail (pl. XIX, fig. 11, A, B) is relatively long. It thins out rapidly and ends into a fine tip that is slightly curved towards the dorsal side. It bears a pair of fine lateral papillae, inserted behind the anus, at a distance one and a half times longer than the rectum. The latter is slightly shorter than the body width at the level of its insertion with the intestine. It is flanked at this point by dorsal and ventral glands.

The intestine, when seen with transmitted light, appears dark greyish and never opaque blackish as in so many other *Rhabditis*. This difference comes from the fact that its cells only contain albumino-fatty granules and never birefringent corpuscles. On this matter, this species resembles *Rhabditis elegans*. Also like the latter, the intestine is composed of two rows of large cells.

The nerve ring (pl. XIX, fig. 10, c), of fibrous structure, envelops the constricted neck of the esophagus exactly above the posterior bulb. It bends obliquely towards the ventral side and extends towards the excretory pore.

The excretory organ is little developed and hard to observe. I only could see with certainty the pore and the unpaired canal that leads into it. They always appeared to me to be located at the level of the junction of the posterior bulb and the intestine.

The vulva is located slightly posterior to the middle of the body length. Its lips barely protrude. The vagina is very short. The paired genital tube is relatively little developed. Its anterior and posterior bends almost reach on both sides the anterior and posterior extremities of the intestine. In contrast, the uteri are very short and never contain more than three or four eggs. The oviduct (tuba) also serves as a seminal receptacle. The vitellogen is very long and may contain eight to ten ova. The ovary, folded in the opposite direction,

often extends beyond the vulva. The germ cells are laid out around a rachis, as in *Rhabditis elegans*. They envelop the rachis as a simple layer that simulates an epithelium.

The eggs (pl. XIX, fig. 12), of oblong shape, rounded at the extremities, measure 60 μ in length and 33 in width. Their shell is thin and smooth.

The male tail (pl. XIX, fig. 13, and pl. XX, fig. 1) terminates in a wide bursa, closed in the front and of the peloderan type, that is, fully enveloping the tail. Seen fullface, it has an elliptical contour that is slightly narrower on the posterior side. Its edges undulate slightly. The nine papillae are arranged as three clearly separate groups, each made of three papillae. Only the anterior group is preanal. The shapes of the papillae are slightly variable, strong and thick. The most massive are, going from front to rear, numbers 4, 6, 7 and 8. The five others are narrower and thinner. The neuromuscular net that runs across them can be distinguished very clearly. An unpaired papilla-shaped protrusion is also found at the anterior edge of the genito-cloacal opening.

The spicules, of a smoky brown color, are thin. They end with a thin tip and are completely independent of each other. The gubernaculum (accessory piece) has a length that is slightly more than half that of the spicules.

The testis does not present anything peculiar to note. The spermatozoa (pl. XIX, fig. 14) are very small. Of a perfectly spherical shape, they measure 2 to 3 μ in diameter. They are made of a clear cytoplasm that envelops a compact nucleus. They are perfectly identical in males and in females.

Rhabditis perrieri is mostly oviparous. I never saw intra-uterine hatchings. The eggs remain in the uteri for some time and are always

laid after the 32-blastomere stage, and never more than five eggs can be found at any time in each uterus; most of the time, there are only two to three.

I did not gather any observations on the number of egg-layings, nor on the lifespan of this species. It is quite agile in its movements.

This species much resembles *Rhabditis elegans*. But it can be distinguished from it by its always smaller size and dimensions; by the thinner and longer female tail; by its narrower mouth with smaller lips; by the overall shape of its bursa, the arrangement and shape of the papillae; finally by the small number of intra-uterine eggs and its essentially oviparous mode of laying. By its size and the conformation of the female tail, one could liken it to *Rhabditis (Pelodera) dentata* of Schneider. But it can immediately be distinguished from it by its buccal cavity.

In order to ascertain the frequency of males, I started four cultures with the methods described for *Rhabditis elegans*. The counting of these four cultures gave me a total of 7,565 females and 55 males: that is 7 males for 1,000 females. But as for the previous species, this average figure may vary from 1 until 10 to 12 for 1,000. These proportions are close to those found for *Rhabditis Marionis*.

As in the previous species, I tried to obtain heterogamous refertilizations, by placing together males with females that had exhausted their own stock of sperm. On two preparations, I thus gathered a total of 26 females and 35 males. These animals remained together, vigorous and healthy, for eight to ten days. However, not a single fertilized egg was laid. One must thus conclude that no mating had occurred, and that the males of *R. Perrieri* have become, regarding sexual matters, as indifferent, if not more, than those of the previous species.

RHABDITIS GUIGNARDI mihi

I found this species only once in soil that was collected at the entrance of the forest of the Reghaia (Algeria). I brought it into culture onto depression slides and multiplied it by feeding it with rotten flesh. I dedicate it to Mr. Guignard, director of the *Ecole supérieure de pharmacie*.

Measurements:

| | ♀ | ♂ | ♂ |
|---------------------|----------|---------|---------|
| Body | 1480 □ | 1072 □ | 858 □ |
| Esophagus | 207=¹/₇ | 171=¹/₆ | 171=¹/₅ |
| Tail | 128=¹/₁₁ | 57=¹/₁₉ | 57=¹/₁₅ |
| Vulva | 772 | | |
| Diameter | 71=¹/₂₁ | 50=¹/₂₁ | 43=¹/₂₀ |
| Buccal cavity | 28=¹/₇ | 23=¹/₇ | 23=¹/₇ |
| Spicules | | 42 | 40 |

The body of the females (pl. XX, fig. 2), cylindrical in its middle part, gradually becomes narrower towards its two extremities. In the front, it is truncated by the mouth; in the rear, it thins out in a fine tip of medium length. The males are always shorter and thinner.

The cuticle is smooth, transparent, colorless and without any apparent transverse striation. The lateral membranes are very narrow and barely protrude.

The mouth (pl. XX, fig. 3) ends the anterior extremity without any narrowing nor protuberance of any sort that would distinguish it from the rest of the body. It is lined by three little protruding bilobed lips. Each lobe bears an extremely fine papilla. – The buccal cavity, of a length of 28 to 30 □, is perfectly cylindrical along its entire length. Its chitinized walls are rather thick. The posterior constriction and bulge are well outlined, yet relatively short.

The esophagus (pl. XX, fig. 4) presents the usual conformations. The anterior bulb has an elongated and slightly spindle-shaped contour. The posterior bulb is more rounded. The valves (teeth) are well developed. In the front, the esophagus sheath extends upwards along the buccal cavity until slightly beyond the extremity of its anterior fourth.

The female tail (pl. XX, fig. 5), of an elongated conical shape, ends in a fine tip. Its narrowing occurs in a regular and gradual fashion. It bears a pair of fine lateral papillae, inserted behind the anus at a distance that equals one and a half times the length of the rectum.

The cells of the intestine, along all its length, contain many birefringent granules. These corpuscles, of spherical shape, may reach diameters of 3 to 4 μ . They are always found in greater quantity in the posterior half of the intestine. The latter however begins and ends with two short sections that are always devoid of granules and as a result, are transparent, whereas the entire rest is opaque blackish. The rectum has a length that is approximately equal to that of the diameter of the body at the level of its insertion with the intestine. At this same point, it is flanked with ventral and dorsal glands, which each contain two small opaque nuclei.

The nerve ring (pl. XX, fig. 4, c), of fibrous structure, embraces the constricted neck of the esophagus above the posterior bulb, by bending over obliquely towards the ventral side and sending extensions towards the excretory pore.

The excretory pore (pl. XX, fig. 4, p), as a result of the fineness of its parts, is quite difficult to see well. The pore and the small unpaired canal are located towards the middle of the posterior bulb. I succeeded in following the lateral branches: the ascending one until beyond the anterior bulb, the descending one until the rectum.

They have the aspect of fine transparent and slightly sinuous canals. Close to and behind the unpaired canal is found a large glandular cell.

The vulva (pl. XX fig. 2, *v*), located towards the middle of the length of the body, does not have protruding lips. The vagina is very short. The paired genital tube is not very developed and its extreme bends are always somewhat distant from the esophagus and the rectum, respectively. The uteri, of short length, do not ever contain more than 6 to 7 eggs each. The oviduct serves as a seminal receptacle. The vitellogen that follows it may contain six to seven ova. The ovary proper, or germigen, folded in the opposite direction, extends until the level of the vulva.

The eggs (pl. XX, fig. 9), of oblong oval shape, measure 50 to 52 \square in their greater diameter and 33 \square in the smaller. The shell is thin, transparent and smooth.

The bursa (pl. XX, fig. 6, A, B) is well developed. I studied it with the greatest care on the two only males that I encountered. It belongs to the leptoderan type, with a free caudal extension. Seen fullface, it has a regular elliptical shape, quite elongated and opened in the front. The caudal extension, in the shape of an awl, has a length approximately equal to half that of the spicules. The number of papillae differed between both males: seven pairs in one, nine in the other. The figure of seven was found for the most vigorous male that measured 1072 \square . In the latter, there was a tendency for the papillae to fuse to each other. Indeed, the two last papillae on the right side were so intimately joined along their entire length that I had some difficulty to recognize their double status. Moreover, on the left side, the second and third, fourth and fifth, were juxtaposed, almost joined. I concluded that, in this male, the figure of seven was

the result of the atrophy of two papillae, most probably the first and the antepenultimate in the front. Nine pairs would be the normal figure in this species, which is corroborated by the second male and by its great resemblance to that of *Rhabditis Marionis*. These nine pairs are distributed into four groups: a first posterior group of three tightly packed papillae; next, a second less tight group of two; then, a third, again not very tight, group of three; finally a last isolated pair in the front. Only the last one is preanal and inserted towards the middle of the spicule length.

The spicules (pl. XX, fig. 7), rather wide and thickset, are slightly stained with a smoky brown. They are entirely independent of each other and end in a rather blunt tip. The gubernaculum (accessory piece) seen from the side, has the aspect of a rod that does not reach half of the length of the spicules.

The testis does not present anything noteworthy. The seminal reservoir of the only two males that were seen contained many spermatozoa. These (pl. XX, fig. 8) were absolutely identical to each other in the males and in the females. When they are not too tightly packed, they adopt a regular spherical shape with a diameter of 5 μ to 5 μ .5. Their cytoplasm is very finely granular, and the nucleus, of a very similar structure, remains completely indistinct, on live as well as dead material. After treatment with acetic acid, it swells like the cytoplasm and remains invisible. This constitutes an interesting characteristic of the species.

Rhabditis Guignardi is essentially oviparous. The eggs are laid as they mature, always before the division to 16 blastomeres. The number of eggs in each uterus never exceeds six or seven. The layings follow each other quite rapidly.

I isolated several females at birth, in order to determine their lifespan and the number of fertilized eggs that they are able to lay. During these cultures, the temperature was 14° C. Incubation within the eggs lasted 34 to 36 hours, growth until first egg-laying five and a half days; the layings of fertilized eggs lasted for a further 7 to 8 days with a maximum of 100 eggs in twenty-four hours, and totals varying between 480 and 560 eggs. Finally they further lived for 12 to 14 days, laying only unfertilized eggs, which makes a total lifespan of 27 to 28 days since the appearance of the egg.

According to the abovementioned figures, this hermaphrodite thus can produce a stock of sperm that is composed in average of 520 spermatozoa. This is a number that exceeds more than two-fold the stock of the previous species.

The movements of *Rhabditis Guignardi* are very slow and heavy. In the cultures, it stays most of the time at the same place, performing only slight torsions on the right and on the left. It is unable to proceed and to exit the waterdrops in which it is reared. Even the male is not very agile.

It is very easy to rear on a depression slide, by feeding it with rotten flesh. But when it approaches the second molt, it passes very easily into the encysted state, so that, in crowded cultures, these arrested larvae are always found in large numbers.

In order to ascertain the existence and frequency of males, I organized many cultures with the devices and methods described for *Rhabditis elegans*. I thus counted 12,657 individuals, among which I only encountered 2 males: that is 999.84 of females and 0.15 of males per thousand. This is by far the smallest proportion that I observed in any species. Here the hermaphroditism has become

almost perfect. The rise in the figure of production of spermatozoa is very probably a fact that points in the same direction.

Rhabditis Guignardi resembles *R. Marionis* so much that we have to enter a minute comparison in order to clearly establish the real differences that separate these two forms. They resemble each other by the shape and the general proportions, by the mouth and the lips, by the female tail, by the intestine and its granulations, by the number of male caudal papillae, by the laying mode and the movements. But *R. Guignardi* is shorter, its buccal cavity is slightly longer, its anterior bulb is spindle-shaped and not rounded, its eggs are shorter, its bursa is not serrated on the posterior side, its spicules are shorter and its spermatozoa smaller and without apparent nucleus. It further differs by the absence of half or fully unisexual females that are so frequent in the other species, by the much higher number of fertilized eggs and finally by the rarity of males.

RHABDITIS VIGUIERI mihi

I found it only once in rich soil collected on the sides of a small lake located at the top of the mountain of Mouzaia (Algeria). I made some cultures of it on depression slides, by feeding it with rotten flesh. I dedicate it to the doctor Viguier, professor at the Faculty of Sciences of Algiers.

Measurements:

| | ♀ | ♂ |
|---------------------|---------|---------|
| Body | 1330 □ | 715 □ |
| Esophagus | 171=¹/₈ | 150=¹/₅ |
| Tail | 275=¹/₅ | 26=¹/₂₇ |
| Vulva | 572 | |
| Diameter | 46=¹/₂₉ | 26=¹/₂₇ |
| Buccal cavity | 20=¹/₈ | 20=¹/₇ |

The body of the females (pl. XX, fig. 10), becoming slightly thicker in the middle, gradually becomes narrower towards its two extremities. In the front, it ends by the truncation of the mouth; in the rear, it thins out in a fine and very long tip. It has a general opaque blackish aspect, caused by the many granulations contained in the cells of the intestine.

The cuticle is very thin, transparent and smooth, without striation nor ornament of any sort.

The mouth (pl. XX, fig. 11) ends the anterior extremity without any protuberance nor narrowing of any kind that would distinguish it from the rest of the body. It is lined by three small bilobed lips, each lobe of which bears a small papilla. – The buccal cavity, long of 20 \square , has the shape of a cylinder with walls that are thickened and slightly flared at the anterior end. The posterior constriction and thickening display the usual configuration.

The esophagus (pl. XX, fig. 12) presents two well-developed bulbs of a slightly ovoid shape. The intercalary neck is relatively thick. The valves (teeth) of the posterior bulb are strong and vigorous. In the front, the esophagus sheath envelops the buccal cavity, extending upwards until its first anterior third.

The female tail (pl. XX, fig. 10) thins out gradually in its first third, then tapers into a long and thin filament. It bears fine lateral papillae, inserted behind the anus at a distance double that of the length of the rectum. These papillae are difficult to see.

The cells of the intestine, besides albumino-fatty granulations, always contain many birefringent corpuscles of spherical shape, which may reach diameters of 4 to 5 \square . These corpuscles give the intestinal tract its opaque blackish aspect. The intestine is composed

of large cells arranged in two rows, as in *Rhabditis elegans*. The rectum, at its point of insertion with the intestine, is flanked with dorsal and ventral unicellular glands. Its length approximately equals the width of the body at the level of its anterior extremity.

The nerve ring (pl. XX, fig. 12, c), of fibrous structure, envelops the narrow neck of the esophagus slightly in front of the second bulb and bends obliquely towards the ventral side, extending in the direction of the excretory pore.

The excretory organ (pl. XX, fig. 12, p) is easy to see. The pore and the unpaired small canal are located at the anterior level of the second bulb. I could clearly follow the lateral canals, the anterior one until the front of the first bulb, the posterior one until beyond the anus.

The vulva (pl. XX, fig. 10, v), which is located quite exactly towards the middle of the intestinal tract, has lips that barely protrude. The vagina is very short. The genital tube, in its whole, is relatively little developed. It is paired and its extreme bends reach quite close to the esophagus in the front and the extremity of the intestine in the rear, respectively. The uteri are short and each never contains more than two eggs at the same time. I have not observed any oviduct that would be morphologically distinct from the uterus, and it seemed to me that the latter continued with the vitellogen without any special narrowing. I do not dare however to state with certainty the absence of a particularly differentiated oviduct, which could well have escaped my notice, compressed as it may between the uterus and the vitellogen. The latter is the most developed part of the genital tube; indeed, one can count along its length as much as twenty ova in the way to maturation. The germigen, folded in the

opposite direction, is very short and only has about a third of the length of the vitellogen.

The eggs, of oblong shape, rounded at the extremities, measure 45 to 55 μ in their greater diameter and 28 to 32 μ in their lesser. The shell is thin and smooth.

The male tail (pl. XX, fig. 13, A, B) widens into a well-developed bursa, opened in the front and of the peloderan type, that is, fully embracing the caudal extremity. Seen fullface, it has an overall elliptical contour, with a slightly serrated posterior edge. The papillae, numbering nine pairs, are arranged in four groups: 1° a terminal group of rather tight papillae; 2° a single papilla; 3° a group of four papillae slightly less tight than the posterior group; 4° finally a last isolated papilla rather far in front at the level of the middle of the length of the spicules. Only this last pair is preanal.

The spicules (pl. XX, fig. 13), rather thin and colorless, are completely independent of each other. They end posteriorly in a fine tip, in the front with a rounded head. The gubernaculum has approximately half of their length.

Rhabditis Vigueri is essentially oviparous. The eggs are laid as they mature, almost always before the third or fourth division of the blastomeres. One can never see more than two eggs in each uterus, and the layings follow each other rapidly.

The development of this species is very rapid. At a temperature of 22° C., eggs laid in the morning hatch in the evening after 12 to 13 hours of incubation. The juveniles that come from these eggs, well fed, laid their first eggs 48 hours later. I have only one observation on the number of eggs that the hermaphroditic females are able to lay. One isolated female laid 150 eggs in a period of 3 days, then died

24 hours later. It thus seems that, whereas in this species growth and multiplication are fast, life on the other hand is quite short.

The movements are rather agile and obtain from rapid undulations.

Unfortunately, I did not make methodically organized cultures in order to determine the proportional frequency of the males. According to an approximate estimation that I find in my notes, this proportion, much higher than in the previous species, could rise from 40 to 50 for 1,000 females. These more numerous males are also more active, and they can often be seen mating. Females that I had isolated when very young and which, not having produced any sperm, were unisexual, were all fertilized by the males that I gave them and laid many fertile eggs from the time of their meeting with these males. I did not keep these eggs originating from a heterogamous fertilization.

Females that are not hermaphroditic but only unisexual are also very frequent. It was indeed sufficient for me to place under the microscope about ten females taken at random to encounter one or two that were unisexual.

In summary, in this species, the still relatively numerous males seem to have kept their sexual instinct intact. The unisexual females are even more numerous than the males, which fertilize them without difficulty. We see here a mixture of hermaphroditism and dioicy, in which all individuals make use of all their reproductive faculties. However, it is again the hermaphroditic state that largely predominates.

RHABDITIS DOLICHURA Schneider

SCHNEIDER. — *Monographie, etc.*, 1866, p. 315, pl. X, fig. 10.

BÜTSCHLI. — *Beiträge für Kenntnis der freilebenden Nematoden*, 1873, p. 115, pl. X, fig 61, *a-b*.

OERLEY. — *Die Rhabditiden, etc.*, 1886, p. 37.

This species is very common, I encountered it several times in soil samples from locations in Algeria that are distant from each other. Schneider and Bütschli found it in Germany, Oerley, in Hungary. I could multiply it at will on depression slides, by feeding it with rotten flesh.

Measurements:

| | ♀ | ♂ |
|---------------------|---------|---------|
| Body | 1000 □ | 672 □ |
| Esophagus | 157=¹/₆ | 128=¹/₅ |
| Tail | 117=¹/₈ | 24=¹/₂₈ |
| Vulva | 495 | |
| Diameter | 59=¹/₁₇ | 30=¹/₂₂ |
| Buccal cavity | 19=¹/₈ | 17=¹/₇ |
| Spicules | | 23 |

The size of the body of adult females varies between 950 and 1,200 □. Its general shape (pl. XXI, fig. 1) is that of a cylinder, slightly swollen in the center, truncated by the mouth in the front and tapering into a conical tip in the rear. Its general aspect is dark greyish rather than opaque blackish.

The cuticle, thin and transparent, bears a delicate transversal striation, sometimes clearly visible, sometimes, on the contrary, invisible. I have actually observed in other Rhabditis such variations in the clearness of the striation. The lateral membranes have the shape of well-marked bands.

The mouth (pl. XXI, fig. 2) is surrounded by six barely-protruding small nipples, which represent the lobes of three serrated lips. Each nipple bears a papilla of extreme fineness. — The buccal cavity, of a

length of 17 to 19 μ , has a perfectly cylindrical shape with thickened walls. The posterior constriction and bulge are well developed.

The esophagus (pl. XXI, fig. 3) is very characteristic, because of the absence of an anterior bulb. The posterior one is rounded and provided with well-developed valves (teeth). The esophagus sheath, in the front, envelops the posterior two thirds of the buccal cavity.

The female tail (pl. XXI, fig. 4) has a rather regular and long conical shape. It bears a pair of lateral papillae of such great fineness that it can only be seen on individuals that present themselves exactly on the ventral side. They are inserted behind the anus at a distance that is equal to two fifths of the rectum length.

The cells of the intestine of well-fed animals contain many albumino-fatty granulations, of shiny and transparent aspect under transmitted light. Beside and mixed with them are always found birefringent corpuscles, which are opaque and yellowish under transmitted light. But these corpuscles are almost exclusively located in the posterior region of the intestine, where they can be found in rather large quantity. These corpuscles have a spherical shape and their maximum diameter never reaches 3 μ .

I checked the structure of the intestine by treating individuals that had been killed with mild heat with 2% acetic acid, and coloring them afterwards with picro-carmine. I could then count more than 80 nuclei along the length of the intestinal tract. They appear (pl. XXI, fig. 5) distributed somewhat irregularly, which makes one tend to believe that the cells to which they correspond have variable shapes and dimensions. The boundaries of the cells are actually difficult to see clearly. But, from the number and packing of the nuclei, it can be concluded that they are arranged in four rows. In *Rhabditis elegans*, which is one third longer, the intestine only totals

30 nuclei, arranged in two rows. Right after hatching, the young larvae only display 16 nuclei that are arranged in two rows along the intestine. At this stage, the intestine thus has the ordinary structure of *Rhabditis*.

The rectum (pl. XXI, fig. 4) is very characteristic for the species by its length and shape and the development of glands that envelop it at its anterior extremity. It is longer of one third compared to the body width at the level of its junction with the intestine.

The nerve ring (pl. XXI, fig. 3), of fibrous structure, envelops the esophagus at the level of the narrow neck in front of the second bulb. It bents obliquely towards the ventral side and sends an extension towards the excretory pore.

The excretory apparatus (pl. XXI, fig. 3, *v*) can only be clearly seen on compressed individuals. The pore is located at the median level of the large bulb. I could follow the lateral branches, the ascending one almost until the buccal cavity, the descending one until the anus. The latter, at its outset, first forms a cluster of irregular and packed meanderings, then follows a perfectly straight course. The ascending branch does not form a cluster, but undergoes weak meanderings along its whole course.

The vulva (pl. XXI, fig. 1, *v*), located rather exactly towards the middle of the body length, has barely protruding lips. The vagina is very short. The genital organ is paired and not very developed, and its extreme bends end at a certain distance of the esophagus, and of the extremity of the intestine, respectively. The uteri are short and no more than 3 to 4 eggs are seen in each of them. The oviduct serves as a seminal receptacle. The little developed vitellogen does not contain more than two to three large ova. The germigen, folded in the opposite direction, is rather thick, but rarely extends until the

level of the vulva. – The eggs, of oblong shape, measure 48 and 26 \square (pl. XX, fig. 14).

On several occasions, I could follow the formation of spermatozoa (pl. XXI, fig. 7, A, B), which precedes the production of ova.

The male tail (pl. XXI, fig. 8, A, B) widens into a somewhat narrow bursa. This bursa, opened in the front, is of the peloderan type, that is fully embracing the caudal extremity. When seen fullface, it has a long elliptical shape that is cut straight at its posterior extremity. The nine papillae are laid out in three clearly distinct groups, each of three papillae: 1^o a very tight posterior group; 2^o a little less tight middle group; 3^o an anterior group with papillae that are rather distant, the last one in the front being inserted at the level of the anterior extremity of the spicules. Only the three anterior papillae are preanal.

The spicules are colorless and independent of each other. They end in a fine tip. They vary somewhat in their shape and their dimensions, and in several individuals, I observed that one of them was shorter than the other. The gubernaculum (accessory piece) has a length of slightly more than half that of the spicules.

The testis does not present anything noteworthy. The seminal reservoir is crammed with small spermatozoa of identical volume and structure to those of the hermaphroditic females. These spermatozoa (pl. XX, fig. 15) are of a perfectly spherical shape, with a diameter of 2 \square . They are composed of a clear and perfectly hyaline peripheral zone and of an opaque central nucleus. I never observed any sign of motility in them.

Rhabditis dolichura is essentially oviparous. The eggs are laid in the order of their arrival into the uteri, and no more than 3 to 4 of them can ever be seen in each of these organs. They are laid during

the first two or three blastomere divisions. Their production is rather fast; a female that I had isolated at a temperature of 25° C. laid hundred of them within twenty-four hours.

I isolated, each separately, three young females, in order to ascertain the number of fertilized eggs that they are able to produce. They respectively laid 193, 202 and 207 eggs, which developed regularly, after which they only produced sterile eggs. It must thus be concluded that the genital organ of these hermaphrodites is able to function as a testis for the production of only 200 to 210 spermatozoa.

The development and lifespan of this nematode are short and fast. At a temperature of 23° to 24° C. the eggs take about eighteen hours to develop until hatching. Forty-eight hours are then required for the juveniles to reach the laying of their first egg. The laying of fertilized eggs lasts for two and a half to three days, after which the mothers can live for a further six to eight days, only producing sterile eggs. The total duration is thus of twelve to fourteen days at most.

Rhabditis dolichura is very agile. It moves quickly in water, undulating like an eel.

It can be easily distinguished from the other hermaphrodites by the conformation of its esophagus without an anterior bulb and by the great length of its rectum.

Wanting to ascertain the existence and frequency of males, I organized and explored mass cultures with the devices and the method that were described concerning *Rhabditis elegans*. These five cultures gave a total of 7,136 individuals, among which I only encountered five males; that is, 0.7 of ♂ for 1,000 ♀. The males are thus very rare. Moreover, their rather small size can easily explain the fact that they escaped the notice of an observer as skilled as

Erlanger¹, who raised more than hundred generations of this *Rhabditis* without succeeding in seeing a single one.

The organization of the few males that I observed appeared to me regular and normal, except for the small irregularity in the spicules, of which one was shorter than the other in two or three animals. The many spermatozoa that filled the seminal reservoir were well conformed and absolutely identical to that of the seminal receptacle of females.

I tried to obtain heterogamous refertilizations by placing together males and females that had exhausted their own stock of sperm. Twelve females and five males were thus put together and lived side by side for six to seven days; but not a single fertilized egg was laid. It must be concluded that the sexual instinct of these males must be deeply atrophied, since none of them tried to mate.

RHABDITIS CORONATA Cobb

COBB. – Nematodes, mostly australian and figian. *Macleay memorial volume*. Sidney, 1893, p. 279, pl. XXXVIII.

DE MAN. – Description of three species of Anguillulidae, etc.

Transactions Liverpool biological society, t. IX, 1895, p. 81,
fig. 2.

This small species appears to be common and distributed more or less everywhere. I encountered it in rich soils coming from the surroundings of Algiers and the high parts (alt. 1,500 m.) of the mountains of the Jurjura. Another time, I found it on a sample of manure coming from Alais, France, Cobb discovered it in the Fidji islands, and De Man in England.

¹ *Biologisches Centralblatt*, t. XVII, 1897, p. 153.

Measurements:

| | ♀ | ♂ |
|---------------------|-------------|-------------|
| Body | 500 □ | 400 □ |
| Esophagus | $100=^1/_5$ | $105=^1/_4$ |
| Tail | $72=^1/_7$ | $28=^1/_14$ |
| Vulva | 264 | |
| Diameter | $33=^1/_15$ | $25=^1/_15$ |
| Buccal cavity | $26=^1/_4$ | $23=^1/_4$ |
| Spicules | | 25 |

The body of the adult females (pl. XXII, fig. 1), cylindrical and relatively thickset, tapers gradually towards both extremities. In the front, it is truncated by the mouth; in the rear, it thins out in a fine tip. It has a general opaque blackish aspect, caused by the many birefringent corpuscles contained in the width of the intestinal cells.

The cuticle, colorless and thick, appeared to me smooth and without striations; but De Man describes a fine striation in the anterior region of the body. He has moreover observed wide lateral membranes.

The mouth (pl. XXII, fig. 2) is not made of fleshy lips, as in the other Rhabditis. It is equipped with two pairs of chitinous appendages, of very different shapes for each pair. The exact conformation of these appendages is difficult to grasp because of their smallness, and like De Man, I do not pretend to have reached a rigorously certain view. A first pair, shorter but thicker and more chitinized, strikes by its opaque blackish aspect. Each appendage appeared to me to have the shape of a wide and irregular hook (see the figure), ending in a rather fine tip. The appendages of the second pair, longer and protruding more towards the front, present the aspect of thin transparent strips, in the shape of a quarter of circle and very finely striated and pectinate on their edge. This description

matches that of De Man for the number of appendages and their division in two pairs differing by their shape.

The buccal cavity is comparatively very long. It indeed equals a quarter (De Man says a fifth) of the esophagus length, measured from the anterior extremity of the mouth. Its shape is that of a regular cylinder with walls that are rather thin than thick. In the rear, it directly connects to the esophagus, without the narrowing and thickening that are usual for *Rhabditis*.

The esophagus (pl. XXII, fig. 3) presents the usual conformation, except for the absence of an anterior bulb. The anterior part of the esophagus bulges gently until the narrow neck, without a distinct bulb. Cobb and De Man, however, mention and describe it as having a barely-bulged, elliptical, shape. The posterior bulb is regularly rounded and its valves (teeth) are well developed. In the front, the esophagus does not extend as a sheath enveloping the buccal cavity as in most *Rhabditis*.

The female tail (pl. XXII, fig. 1), starting from the anus, tapers gradually to thin out in a fine tip.

In addition to the albumino-fatty granulations, the cells of the intestine always contain many birefringent granules, of spherical shape and rather voluminous. These corpuscles give the intestine its opaque blackish aspect. – The rectum has at the level of its insertion with the intestine a length equal to the body width. The rectal glands, one dorsal and one ventral, are well developed.

The nerve ring (pl. XXII, fig. 3, c), of fibrous structure, envelops the neck of the esophagus slightly above the second bulb. It bends obliquely towards the ventral side and sends out extensions towards the excretory pore.

The excretory organ (pl. XXII, fig. 3, *p*) is very difficult to see. I only succeeded in distinguishing the pore and the unpaired canal that leads to it, which are located at the level of the posterior bulb; I could not see anything of the lateral canals.

The vulva (pl. XXII, fig. 1, *v*) is located rather exactly towards the middle of the total length of the body, its lips are clearly thickened. The vagina is short. The paired genital tube (pl. XXI, fig. 9) is relatively little developed, its extreme bends end at a large distance from the two extremities of the intestine. The uterus is wide and alone makes almost half of the genital tube. Nonetheless, it only ever contains one egg, and the two uteri always alternate, one being empty while the second is filled. At its anterior extremity, it forms a diverticulum with the shape of a sac serving as seminal receptacle. The vitellogen and germigen merge, and only a single maturing ovum can ever be seen. The folded part of the ovary is very short and appears like the hook of a crook.

The eggs (pl. XXI, fig. 10), of oblong shape, measure 52 \square in length and 20 \square in width. They are regularly rounded at the extremities, which sometimes appear slightly bulged. The shell is thick and bears fine little spikes.

The male tail (pl. XXI, fig. 11) belongs to the peloderan type (De Man says leptoderan), that is, the bursa fully envelops the caudal extremity. The base is narrow and opened in the front. The papillae, numbering 7 pairs, are spaced apart rather regularly, without forming any particular groupings. Only two are preanal and the last one in the front is inserted at the level of the middle of the length of the spicules.

The spicules (pl. XXI, fig. 11), of a dark brown color, are arched and thickset. In the rear, they end in a fine tip, in the front by a

clearly narrowing head. They are independent of each other. The gubernaculum (accessory piece) is thick and equals in length half that of the spicules.

The testis does not present anything noteworthy. The spermatozoa, whether of masculine or of feminine origin, are perfectly identical to each other. Of spherical shape and very small, they measure 1 \square to 1.5 \square . Their substance appears to be reduced to the nucleus only and their aspect is homogeneous.

Rhabditis coronata is essentially oviparous. In the uteri, there is only a single mature egg at a time, alternatively in each uterus. The eggs do not remain in the uterus but are laid as soon as the shell is developed and before the division into two blastomeres. The layings follow each other slowly. At a temperature of 21° C., an isolated female laid only six eggs in 24 hours. These eggs, at the same temperature, take three full days to develop until hatching.

I have not gathered any observations on the total number of layings, nor on the length of the larval growth and lifespan.

The movements of this nematode are very slow and heavy. In the waterdrops in which it is raised, it wriggles about slowly by contorsions, but without moving forward nor circulating.

On several occasions, I isolated juveniles before adulthood, in a total of about 150 individuals. All became females, which laid fertile eggs that developed regularly. When examined under the microscope at high magnification, all these females presented a seminal receptacle that was stocked with small spermatozoa identical to those of the males. No doubt can therefore remain: the species is protandrous autogamous hermaphroditic.

I did not make any methodical cultures to ascertain the frequency and the ratio of males. However, from approximations found in my

notes, I believe that I can state that no more than 5 to 6 per 1,000 individuals can be found. It is very probable that, here as in the previous species, these rare males do not play any role in the reproduction of the species. But I have not performed any experiment regarding this point.

Cobb does not even mention the existence of males. De Man says to have encountered a single one, among a very large number of females.

DIPLOGASTER ROBUSTUS mihi

I found this species only once, in black soil sampled under the pinetrees of the higher part of the experimental garden at the Hamma near Algiers. I could make it multiply at will on depression slides, feeding it with rotten flesh.

Measurements:

| | ♀ | ♂ | YOUNG ♀ with a single egg in the uteri | YOUNG at hatching |
|---------------------|----------|---------|--|----------------------|
| Body | 2488 □ | 1400 □ | 1387 □ | 382 □ |
| Esophagus | 257=¹/₉ | 200=¹/₇ | 200=¹/₇ | 112=¹/₃₄ |
| Tail | 314=¹/₈ | 143=¹/₉ | 214=¹/₆ | 66=¹/₆ |
| Vulva | 1244 | | 672 | |
| Diameter | 128=¹/₁₉ | 71=¹/₁₉ | 71=¹/₁₉ | 19=¹/₂₀ |
| Buccal cavity | 13=¹/₂₀ | 10=¹/₂₀ | 12=¹/₁₇ | 5=¹/₂₂ |
| Spicules | | 47 | | |

The size of adult females can vary from 1,390 □ to 2,490 □; that of the males from 1,320 □ to 1,400 □.

The body of females (pl. XXII, fig. 4), thick and robust, is slightly bulged in its middle part. It tapers gradually and slowly towards the extremities. In the front, it is truncated by the mouth; in the rear, it thins out in a fine tip. Its general aspect is opaque blackish, because of the many granulations of the intestine.

The cuticle is colorless, transparent and rather thick. It is decorated with fine longitudinal veins that are characteristic of *Diplogaster*. These veins, numbering 34 to 36 in the middle region, are composed of two rows of fine punctuations (pl. XXII, fig. 5). In addition to these longitudinal veins, an extremely delicate transversal striation, with one stria for two punctuations of the veins, can also be perceived in favorable situations. These striata, at the crossing of the veins, also resolve into two or three very small punctuations.

The mouth (pl. XXII, fig. 6) ends the anterior extremity without protuberance or constriction of any sort that would distinguish it from the rest of the body. It is lined by six small and little-salient lips, each equipped with a fine papilla. The buccal cavity, wider than deep, has rather thick chitinized walls. Two little-salient conical teeth are found at the bottom.

The esophagus (pl. XXII, fig. 7) presents the usual conformation. The anterior half is of one third longer than the posterior half. The anterior bulb, of a slightly elongated shape, is well developed. Its internal valvular walls are strongly chitinized. The rounded posterior bulb always displays two large clear and nucleolated nuclei within its fleshy substance.

The female tail (pl. XXII, fig. 8), starting from the anus, thins out gradually in a long, thin and tapering cone. It bears a pair of fine lateral papillae that are rather difficult to see and are inserted behind the anus at a slightly longer distance than the length of the rectum.

The intestine, in well-fed individuals, always has an opaque blackish aspect, caused by the many granulations that accumulate and are stored in the constituent cells of its wall. Some of these

granulations are of albumino-fatty nature; the others with a crystalline birefringent structure. The latter can be very abundant, and they are mostly responsible for the great opacity. The rectum has at the level of its junction with the intestine a length equal to the body width. At this junction, it is endowed with well-developed dorsal and ventral glands.

The nerve ring (pl. XXII, fig. 7, c), of fibrous structure, envelops the narrow neck of the esophagus above the second bulb, bending obliquely towards the ventral side and sending out extensions towards the excretory pore.

The excretory organ (pl. XXII, fig. 7, p) is easy to distinguish. The pore and unpaired canal are located at the posterior level of the second bulb. The anterior lateral branching is very sinuous, with short sinuosities; it can be followed without effort beyond the front end of the anterior bulb. The posterior canal is almost straight and I could follow it until the vicinity of the rectum. At the point of merging of these canals with the unpaired canal are found two unicellular glands, each provided with a large and clear nucleus with an opaque nucleolus.

The vulva (pl. XXII, fig. 4 and 9, v) has the shape of a round and narrow opening, without protruding lips. It always opens on one of the longitudinal veins, the diameter of which it barely exceeds when closed. The vagina is relatively developed and presents the aspect of a narrow tube, directed in the transversal direction within the body.

The paired genital tube is greatly developed (pl. XXII, fig. 4 and 9), its extreme bends come quite close to the two extremities of the intestine. The uteri are wide and spacious; they continue into a narrow oviduct (tuba). The latter, in the very young females (pl. XXII, fig. 9) that still have only one or two eggs in each uterus, runs

directly without sinuosities until the extremity of the ovary, to which it connects by making its extreme bend. But in the older females (pl. XXII, fig. 4) whose uteri are crammed with eggs, the oviduct, being held back by the bloated uterus, folds on itself. There is no special pocket serving as a seminal receptacle. The spermatozoa are stored (pl. XXII, fig. 9) in part in the uterus, and in part in the ovary, at the junction points of these two organs with the oviduct. The ovaries proper do not form bends, but directly extend along the length of the body, simply orienting obliquely across it, in order to fit alongside the intestine. The free extremity of each of them extends largely beyond the vulva and almost reaches the head of the opposite ovary. The distinction between the germigen and the vitellogen can only be made by the development of about twelve big ova in the latter.

The eggs (pl. XXII, fig. 10), of an oblong shape rounded at the extremities, measure 79 \square in length and 46 in width. Their shell is fine and smooth.

The male tail (pl. XXIII, fig. 1), in its general conformation, completely resembles the female tail. It extends and gradually tapers into a long and thin cone. It is provided with a small, narrow and little-protruding bursa, which extends in the front and in the rear of the anus at distances that exceed somewhat the length of the spicules. There are 10 pairs of papillae: first, completely in the rear towards the extremity of the first third of the tail, three small papillae in a tight group, inserted onto the ventral side of the tail; then three other stronger papillae, laterally inserted between the anus and the previous ones and consequently postanal like the latter; the two next ones inserted very closely in front of the anus, laterally one above the other; finally number 9 at the level of the anterior extremity of the spicules and number 10 still further

anteriorly, at a distance equal to the length of the spicules. All these papillae, save for the three small posterior ones, are strong, conical and clearly show a central neuro-muscular net. It seemed to me that in addition to these ten paired papillae, there was still an unpaired one on the anterior lip of the cloacal opening.

The spicules (pl. XXIII, fig. 2), thin and very arched, are slightly colored with a smoky brown. They end in the front by a rounded head, in the rear in a fine tip. The gubernaculum (accessory piece) equals in length the third of the spicules. Very thin in the rear, it suddenly thickens in its anterior third. – In one of the only three males that I encountered, the spicules presented irregularities, one being one third shorter than the other, and of somewhat anomalous shape. We already noted above analogous anomalies in the males of *Rhabditis dolichura*.

The testis is conformed according to the usual type in rhabditids. In one of the males, which I kept alive for eight days during its full maturity, it was transformed into a vast seminal reservoir, from the vas deferens until almost the anterior bend. This entire length was packed with spermatozoa that were piled upon each other. Next to the bend, the spermatoblasts still continued to transform themselves into spermatozoa, dividing twice. In the middle of this mass of spermatozoa were irregularly scattered many crystalline needles (pl. XXII, fig. 11) of a length of 14 to 50 μ . These needles sometimes end in a fine tip, sometimes are truncated as if broken. They had no morphological relationship to the spermatozoa and had doubtless formed there following some chemical reaction. They probably represent a residual substance that has accumulated in the seminal reservoir. When treated with acetic acid, they dissolved without

leaving any trace. There were similar ones in the testes of the other two males. Their presence thus appears constant in this *Diplogaster*.

I had the opportunity to see similar needles in the seminal reservoir of *Rhabditis teres*, where Bütschli¹ had also observed them. Claus, before him², had already described identical ones in his *Rhabditis brevispina*. These two scientists consider these productions to be a particular form of spermatozoa. But I am convinced that their interpretation is erroneous.

The spermatozoa (pl. XXII, fig. 12), whether of feminine or of masculine origin, are identical to each other. Of spheroidal shape, they measure 6 to 7 \square . They appeared to me to be a little smaller in females than in males. When not deformed by compression, they always take a regular spherical shape. I never saw them display the least contractile or motile ability. In the living state, their nucleus is barely apparent. But when observed on males or females that were killed by acetic acid and cleared by this reagent, it very clearly appears to be composed (pl. XXII, fig. 13) of twelve small spherical elements or chromosomes. I counted these corpuscles in about forty spermatozoa of masculine or feminine origin, and always found 12 of them.

This resistance to the swelling action of acetic acid is not found in the nucleus of spermatoblasts, which, under the influence of this reagent, become so transparent that they disappear from sight. The same holds true for the germinal nuclei of ova. Thus, it is at the time of the final formation of the spermatozoon that the nuclear elements take this aptitude and acquire this peculiar structure. Regarding this point, they completely recall what occurs in the micronucleus of

¹ *Beiträge zur Kenntnis der freilebenden Nematoden*, 1873, p. 100 and 110, pl. XI, fig. 64, h.

² *Zeitschrift für wiss. Zoologie*, t. XII, p. 357, pl. XXXV, fig. 4².

many ciliated infusoria, which I many times had seen with the help of this same reagent.

The eggs of this *Diplogaster* are not laid as they mature; they remain and are stored in the uteri where they continue to develop. They are always laid quite late, even often when the embryo is almost completely formed. At the beginning, all the products are laid as eggs. But towards the end of the fertile period, intra-uterine hatchings become frequent, and many mothers die being devoured by their progeny. In two females, I counted a total of 76 to 78 eggs held together in the uteri, the oldest ones with young embryos ready to hatch, the most recent ones at the two and four-blastomere stage.

Several times, I isolated non-adult females, in order to ascertain the number of fertilized eggs that they are able to lay. The figures I obtained varied much more than in *Rhabditis* and oscillated between 140 to 230 eggs. They correspond to the number of spermatozoa that the genital gland is able to produce when functioning as a testis.

This *Diplogaster*, at a temperature of 20° C., can live for 16 days; but the individuals that succeed in reaching this maximum are rare. The large majority indeed perishes much earlier, following intra-uterine hatchings. The eggs require 12 hours for their embryonic development. The laying of the first egg happens 3 days later; the fertile layings last for 3 to 3 and a half days: then, if intra-uterine hatchings did not result in its precocious death, the animal continues to lay unfertilized eggs for 6 to 8 days, gradually weakening, to end of senile exhaustion.

The juveniles that have just hatched (pl. XXIII, fig. 3) measure 380 to 390 μ . They already possess all their organs, except for the genital apparatus that is still in a very rudimentary state. The mouth (pl. XXIII, fig. 4) differs quite notably from the adult form. It is

narrower and relatively deeper. Its structure also appears simpler. The proportions of the parts of the body are also quite different. The esophagus and the tail represent $1/3$ and $1/6$ of the total length, respectively, instead of $1/9$ and $1/8$ in the adult. It results thereof that in the final growth, the greatest part is contributed by the median intestinal region which multiplies 8-fold, whereas the esophagus and the tail only multiply 2 and 4-fold, respectively. The intestine is composed of 17 to 18 cells, arranged in two rows. The genital primordium (pl. XXIII, fig. 5), of oblong elliptical shape, is composed of two large nucleolated germinal nuclei, with a small somatic cell at each pole, the whole being enveloped by an anhistic membrane.

This species frequently encysts. I did not observe its molts. Its movements are slow and heavy.

There is a great kinship and a rather great resemblance between our *Diplogaster robustus* and *D. longicauda* described by Bütschli¹ and by Ziegler², which is actually not at all identical to the *D. longicauda* of Claus. But ours differs from that of Bütschli by its size and the much greater proportions of its diverse parts, by its hermaphroditic state and finally by having 10 male papillae, whereas the other only has 9.

In order to ascertain the existence of males and evaluate their frequency, I organized large cultures that I arranged and explored as described above concerning *Rhabditis elegans*. I studied in this manner 4 series of layings, in each of which all fertile products of 40 females were brought to the adult stage. I thus counted 23,445 individuals, among which I only encountered 3 males; the only ones that I ever saw in this species, which were used for the description

¹ *Zeitschrift für wiss. Zoologie*, t. XXVI, 1876, p. 369, pl. XXIII, fig. 1, a-c.

² *Zeitschrift für wiss. Zoologie*, t. LX, 1895, p. 355.

given above. This is a proportion of 0.13 ♂ for 1,000 ♀, by far the smallest that we know of. In this *Diplogaster*, hermaphroditism has become almost absolute, even though the production of spermatozoa by females is relatively low. I never noticed any fact that would have indicated the existence of non-hermaphroditic females, laying unfertilized eggs from the beginning.

With two of the males, I tried to obtain heterogamous refertilizations, isolating each of them separately with two young females that had just undergone their fourth molt, that is, were entering the adult age. These animals lived side by side for 7 to 8 days, being vigorous and healthy. With one of the males, I did not see any attempt at mating; with the second, on the contrary, I saw him twice holding a female embraced in its caudal extremity, which was folded in the attitude of mating. The 4 females laid fertilized eggs for 3 to 4 days, then sterile eggs. The total number of fertilized eggs rose to 581, that is an average of 145 per female, a minimum figure for the layings of autogamous origin. These eggs were all kept and their products raised until the adult age. Thereof 581 hermaphroditic females came out, as in ordinary cultures.

Of all this, one must conclude that no heterogamous refertilization had occurred. The male, enlacing a female with its caudal folds, only performed an enactment of mating that was not followed by actual fertilization. These males, like those of the *Rhabditis* described above, have lost their reproductive sense and only exist as atavistic manifestations of an ancient dioic state. Their role in the reproduction and conservation of the species is absolutely null.

DIPLOGASTER MINOR mihi

This species appears widespread and common. I found it for the first time in a fat soil sampled in Vire in Normandy, then a second and a third time in soils from the South-Oran region (well of Galloul), and the forests of Edough, next to Bône. It is raised and multiplies without difficulties on depression slides, by feeding it with rotten flesh.

Measurements:

| ♀ | YOUNG at hatching |
|---------------------|----------------------|
| | - |
| Body | 1072 □ |
| Esophagus | 143=¹/₇.₅ |
| Tail | 214=¹/₅ |
| Vulva | 500 |
| Diameter | 70=¹/₁₅ |
| Buccal cavity | 11=¹/₁₃ |
| | 12=¹/₂₃ |
| | 4=¹/₂₀ |

The size, in this species, is very uniform and appeared to me to only vary within very restricted boundaries, approximately from 950 to 1,070 □.

The body of the adult females (pl. XXIII, fig. 6), relatively thick and swollen in the center, has a very elongated, spindle-shaped, general aspect. It tapers gradually towards the extremities, truncated in the front by the mouth, thinning out in a fine tip in the rear. Its general color is opaque blackish, due to the many granulations of the intestine.

The cuticle, as in the other *Diplogaster*, is decorated by longitudinal veins that are delicate and somewhat difficult to see. The small bands inbetween the veins are transversally striated.

The mouth (pl. XXIII, fig. 7) is not differentiated from the rest of the body by any constriction or protuberance of any sort. It is lined

by three barely protruding lips, each bearing two extremely fine papillae. The buccal cavity is rather short and approximately as wide as it is deep. Its chitinous walls are thick and formed by two superposed pieces. At the bottom are found two barely protruding teeth.

The esophagus (pl. XXIII, fig. 8) presents the usual conformation. It is relatively wide and thickset. Its anterior half is longer of two thirds than the posterior half. The well-rounded anterior bulb has internal valvular walls that are strongly chitinized. The posterior bulb, also rounded, always shows within its fleshy substance two large clear nuclei with opaque nucleoli.

The tail (pl. XXIII, fig. 9) thins out regularly starting from the anus, to end in a fine tip. Its length may vary two-fold, without modifications in the proportions of the other parts of the body. I did not distinguish any lateral papillae.

The cells of the intestine of well-fed individuals are filled with many albumino-fatty granulations and as many birefringent corpuscles. Mostly the latter give the intestinal tract its opaque blackish aspect. The rectum has at the level of its junction with the intestine a length that is close to the body width. At this junction, it bears clearly visible dorsal and ventral glands. The anus has its posterior lip that bulges out slightly.

The nerve ring (pl. XXIII, fig. 8, c), of fibrous structure, envelops the narrow neck of the esophagus inbetween the two bulbs and bends obliquely towards the ventral face and the excretory pore.

The excretory organ (pl. XXIII, fig. 8, p) is difficult to see well. The pore and the unpaired canal can be distinguished without too much trouble at the level of the middle of the posterior bulb; but I was not able to see the longitudinal canals.

The vulva (pl. XXIII, fig. 6 and 10, v) is rather exactly located in the middle of the body length. It does not form any protuberance and its opening has the shape of a circular pore. The vagina is short. The genital tube, quite strongly developed in some of its parts, has its extreme bends quite far from the two extremities of the intestine. The uteri are narrow and short; each of them never contains more than two or three eggs at the same time. The anterior extremity, at the junction with the oviduct, serves as seminal receptacle. The narrowing region of the oviduct is also quite short. The ovary proper is, in contrast, very developed. It starts at each extremity by forming a hook, then bends and continues obliquely towards the intestine and extends beyond the vulva until the head of the opposite ovary. Its anterior portion containing 7 to 8 large growing ova can be considered as the vitellogen, the rest as the germigen.

The eggs (pl. XXIII, fig. 11), of oblong shape, rounded at the extremities, measure from 60 to 70 μ in length and 33 to 39 μ in width. Their shell is thin and smooth.

Diplogaster minor is essentially oviparous. The eggs are often even laid before their first cleavage, at the latest after the second or third. Thus no more than two or three eggs can ever been seen at the same time in each uterus.

The larvae of this species encyst easily, when they encounter bad nutritional conditions.

The movements are slow and heavy.

This species, in its general conformation, resembles the *Diplogaster robustus* described above. But it can be distinguished from it by its much smaller size and dimensions of its different parts, and above all by the weak development of its uterus, as well as its essentially oviparous state, which is a consequence thereof. It much

resembles *Diplogaster longicauda* of Bütschli and Ziegler that was mentioned above. This resemblance is so great that, if it were not for their sexual dissimilarity, one would fuse them without hesitation. Moreover, I many times tried to cross them, placing males of *D. longicauda* with females of *D. minor*. These attempts remained unfruitful. These two forms do not mate and fertilize each other. They must thus be considered as distinct species.

I ascertained with a single female only the number of fertilized eggs that this species is able to lay. This female, isolated at hatching, laid 261 fertilized eggs. It must be concluded that the genital glands of *D. minor* can produce as many as 261 spermatozoa while functioning as testes. This figure (maybe a maximum?) is slightly higher than that of *D. robustus*.

In order to know the length of its existence, I isolated a juvenile at the time of hatching and followed it until its death. The temperature was 17° C. Growth lasted four days. Then the adult female laid fertile eggs for four days, with daily numbers of 42, 72, 85 and 62, total 261 eggs. Afterwards, she remained for two days with completely empty uteri, not laying anymore. Then the next day, I found it surrounded by unfertilized eggs of a particular nature and to which we shall return below. She continued to lay unfertilized eggs for a further four days, then lived for one day being completely sterile and perished from old age.

In summary, this female lived for a total of 16 days. Since this species is not exposed to mortality caused by intra-uterine hatchings, it is highly probable that its lifespan is approximately the same in all individuals, only varying with temperature.

The fertilized eggs that this female started laying at the 11th, 12th and 13th days of its existence, after a complete rest of two days, these

eggs were very interesting to observe. Some of them, devoid of a shell, disorganized quite rapidly, as we saw for similar eggs in all the other hermaphrodites. But other eggs, in contrast, were equipped with clearly differentiated shells. Some of them, ill-conformed, had extensions of irregular shape (pl. XXIII, fig. 12). Their vitellus was amorphous and appeared disorganized. Finally, some others (pl. XXIII, fig. 13) had even more clearly differentiated shells, with a regular shape, only narrower and thinner than the normal form. Inside, the vitelline mass was cleaved into a certain number of blastomeres that presented the aspect of a morula. But these blastomeres were of a much too regular spherical shape, a hint of a developmental arrest and of a beginning of disorganization. These eggs were kept and found completely disorganized on the next day. The blastomeres only formed a granular amorphous mass that filled the whole shell cavity, as in the egg in figure 12.

These eggs, quite certainly unfertilized, which nonetheless secrete a complete regular shell and can then go through the first cleavage stages, must be considered as eggs on the way to parthenogenetic development, it seems to me. When parthenogenesis develops in a species, it quite certainly does not appear suddenly and all at once. It must be gradually realized, first in imperfect products, such as the eggs that we just studied. It is also probable that it appears and develops mostly, if not always, in protandrous autogamous hermaphroditic species.

I have not encountered a single male in this species. If they exist, they are probably as rare as its previous congener. Regarding this point, I only counted and explored a culture that was composed of 1,796 individuals that were all females.

RHABDITIS SCHNEIDERI BÜTSCHLI

SCHNEIDER. – *Monographie der Nematoden*, 1866, p. 321.

BÜTSCHLI. – *Beiträge*, etc., p. 116, pl. X, fig. 63, a c e, and XI, fig. 63, b.

This species appears to be common and very widespread. Schneider and Bütschli found it in Germany. I encountered it three times in Algeria: twice in the surroundings of Algiers, the third time in the forest of Edough, next to Bône. In each of these sites, it lived in soils that were light, not very rich and not too wet. It does not seem to like the very rich humus that is sought after by the other Rhabditis. It lends itself quite well to culturing on depression slides, feeding it with quite diluted rotten flesh.

The description and the figures that were given by Bütschli being sufficient to recognize it, and not having myself made a complete morphological study of it, I will satisfy myself with presenting the observations that I could gather on its biology and its sexuality.

This species has a very fast development and a short existence. Its eggs undergo their embryonic development in 19 to 20 hours at a temperature of 12 to 13° C., and in 10 to 12 hours only at a temperature of 19 to 20° C. With 12 to 13° C., the larvae need 68 to 70 hours to grow and reach their complete development until the laying of their first egg. This growth only lasts 40 hours at 19 to 20° C. The layings last for 10 to 12 days at 12 to 13° C., and for 8 to 9 days at 19 to 20° C. Once layings are over, the animal lives for a further one or two days, then dies. In summary, the total lifespan is 18 to 19 days at a temperature of 12 to 13° C., and 8 to 9 days with 19 to 20° C.

A well-fed female lays 340 to 380 eggs. When it is fed with albumin, this figure is reduced by more than two thirds. The

maximum number of eggs laid in 24 hours can be of 35 to 40 eggs at 12 to 13° C., and of 130 to 140 at 19 to 20° C.

Schneider and Bütschli expressly state that they only saw females in this species. I wanted to ascertain this absence of males by using the same research methods that I described above concerning *Rhabditis elegans*. I thus reared and counted 4,039 adult individuals, all females without a single male. If this sex ever exists, it must be very rare.

As we said in the historical part of this work, *Rhabditis Schneideri* is the first case of well-recognized parthenogenesis that was ascertained in nematodes. The two German scientists, without using the word parthenogenesis, very clearly state that it reproduces without fertilization. They could never see a male nor the least sign of spermatozoa in the genital organs of females that were laying many regularly developing eggs.

Although the authority of these two skilled observers was perfectly sufficient to give this fact a definitive authenticity, it has remained effectively unknown to science. We saw in the historical introduction that it was not taken into account. Therefore, it appeared to me useful to verify it again and to ascertain it. We thus examined with the greatest care, with the help of the best objectives and using diverse methods, the genital organs of many females, without ever seeing the least sign of spermatozoa in them. This survey is actually facilitated by the clearness of the organs, especially when one takes the care of starving the animals for a while before using them. One can then, with a little compression, minutely explore the genital organs in all their parts. Thus not the least doubt can remain today, *R. Schneideri* is a species with parthenogenetic reproduction.

CEPHALOBUS DUBIUS mihi

This species is very widespread and common in Algeria. I encountered it several times in the surroundings of Algiers. It was brought to me from the South-Oran region (Aïn-Sfissifa, Chott-Chergui, Haci-Morra and Taouessera) by Mr. Flamand, from the southern slope of the Jurjura (1,500 meters in altitude) by Mr. Ficheur, finally from Bousaada and its surroundings by the lieutenant Pouget. I further found it in a sample of red soil collected in the surroundings of Antananarivo and sent from Madagascar by Dr. Martel. In all these places, it lives in rather poor soils and can withstand long dessications and revive as soon as it is moistened. I have cultured it for a long time on depression slides by feeding it with albumin.

Measurements:

| | ♀ | YOUNG at hatching |
|---------------------|----------|----------------------|
| Body | 730 ♂ | - |
| Esophagus | 160=¹/₄₆ | 89=¹/₂₇ |
| Tail | 43=¹/₁₇ | 26=¹/₉ |
| Vulva | 473 | |
| Diameter | 50=¹/₁₅ | 14=¹/₁₇ |
| Buccal cavity | 17=¹/₉ | |

The size of the individuals varies between 600 to 730 ♂.

The body (pl. XXIV, fig. 1) is relatively heavy and thickset. In the front, it tapers gradually for a rather long distance in the esophagus region, and ends by the truncation of the mouth, the width of which hardly equals a fourth of the body diameter in its middle part. In the rear, it thins out rapidly starting from the anus, and ends in a very short fine tip in a race (pl. XXIII, fig. 16) that we will call var. *apicata*, and in a rounded and thick tip in the other race (pl. XXIV,

fig. 2) or var. *rotundata*. In all well-fed individuals, its general aspect is opaque, without being blackish as in *Rhabditis*. This opacity comes from the many granulations that are deposited in the walls of the intestine and in the connective tissue. The tail and the esophagus region are always clearer.

The cuticle, thin and transparent, is transversally striated; but the striations are not very apparent and are difficult to see. Often they are searched in vain. When they can be seen, it is only in an optical section at the edges of the observed individual. These edges then appear very finely festooned.

The mouth, in the race *apicata* (pl. XXIII, fig. 15), ends the anterior extremity by smoothly rounding up, without forming a protuberance nor a constriction of any sort that would distinguish it from the rest of the body. It is lined with three little-protruding lips without papillae. In the *rotundata* race (pl. XXIII, fig. 14), the lips are much more developed and separated by strong serrations, and they form a slight terminal bulge protruding from the extremity of the body. These lips do not have the regular rounded shape of the first race, but are rather slightly angular. – The buccal cavity displays the ordinary chitinous thickenings of the *Cephalobus* genus. It has 1/9 of the length of the esophagus measured from the anterior extremity of the mouth.

The esophagus (pl. XXIV, fig. 1, o) has a very elongated and swollen anterior part, which forms a sort of bulb with the shape of a long and rather thin spindle; the narrow neck is short; the second bulb, slightly longer than wide, is equipped with well-developed valves (teeth). In the front, the esophagus extends along the buccal cavity, enveloping it like a sheath until the anterior end of its middle thickening.

The tail, in the *apicata* race (pl. XXIII, fig. 16), is of conical shape, thins out regularly from the anus and ends in a very short and fine tip. In the *rotundata* race (pl. XXIV, fig. 2), in contrast, it rounds up suddenly in a large obtuse tip at its extremity, where it retains a diameter that is still equal to a third of that of the body at the level of the anus. Fine lateral papillae are found inserted behind the anus at a slightly greater distance than half of the rectum length.

The intestine is composed of two rows of alternating cells. In well-fed individuals, these cells are filled with albumino-fatty granulations: birefringent corpuscles can never be found in them as in *Rhabditis*. The granulations also accumulate in great quantity in the connective tissue between the outer wall of the digestive tube and the muscular-cutaneous layer. This connective tissue thus plays a completely analogous role to that of the fat body of insects. These granulations may accumulate up to the anterior region of the esophagus, as well as down to the tip of the tail. By their great abundance, they make this *Cephalobus* so opaque that the study of internal organs becomes highly difficult. The rectum has a length equal to three fourths of the body width at the level of its connection with the intestine. At this connection, it is equipped with a dorsal gland.

The nerve ring (pl. XXIV, fig. 1, c), of fibrous structure, envelops the narrow neck of the esophagus in front of the second bulb. It bends obliquely towards the ventral side and sends out extensions in the same direction.

The excretory organ (pl. XXIV, fig. 1, p) is very difficult to see, and this only on emaciated and quite compressed animals. The pore is located at the anterior level of the second bulb. The unpaired canal describes one or two sinuosities that are somewhat variable from

one individual to the next. I did not succeed in seeing the posterior lateral branch, whereas I could follow the slight sinuosities of the anterior one until the middle of the esophagus. Close to and behind the pore is found a unicellular gland.

The vulva (pl. XXIV, fig. 1, *v*) is located at a slightly variable position, oscillating sometimes slightly anteriorly, sometimes slightly posteriorly of the boundary between the second and the last thirds of the total length of the body. Its lips are slightly protruding. The vagina is very short. The genital tube is unpaired, as in most *Cephalobus*. The uterus extends in front of the vulva on a length equal to twice the body width. Its shape is that of an oblong pocket, terminated by a dead end in the front. It connects to the ovary through a narrow oviduct (*tuba*), which is inserted slightly behind its anterior extremity. This oviduct extends by turning obliquely towards the front to reach the anterior extremity of the ovary, located at the same level as the extremity of the uterus. The ovary then extends directly towards the rear, always making a very pronounced double bend slightly beyond the vulva. It always ends rather far away anteriorly from the extremity of the intestine. The uterus only ever contains a single egg, which is always laid before the next one comes and replaces it.

The transfer of the eggs from the ovary into the uterus proceeds very slowly. They can then be seen entering into the narrow strait of the oviduct, which gives in hardly, and across which they stretch as if in a screwing die. When one half has already passed through and the other still remains in the ovary tube, they then quite well resemble the two halves of a haltere, joined by their handle. Their substance must possess a rather strong tenacity and ductility in order to resist, without falling apart, to the tractions and compressions that it

suffers in this narrow passage. The eggs secrete their solid shell after their arrival into the uterus. Once laid, they have an oblong shape rounded at the extremities, with lengths varying from 53 to 64 μ , and widths from 26 to 30 μ .

This species is very inert. It constantly stays almost motionless, only carrying out slow and rare undulations, which hardly make it move along. When well fed, it remains for entire days at the same position.

I have hesitated for a long time to know whether I should assimilate the form that I just described to the *Cephalobus nanus* of De Man¹. But after a thorough examination, I was led to distinguish them as distinct species. *C. nanus* is smaller by one third; the anterior swelling of its esophagus is more marked; it has the mouth of our race *apicata* and the tail of our race *rotundata*. The genital organ is not sufficiently studied to be able to establish comparisons. De Man has seen no male. If a more complete study of this type would later demonstrate its parthenogenetic state and a sufficient resemblance, so that it would be necessary to assimilate it to my *C. dubius*, it would obviously constitute a third race of this species; which actually seems to have a strong tendency to form local varieties.

Cephalobus dubius is essentially oviparous. Its eggs are always laid before even the first cleavage. A single egg can ever be seen in the uterus and this egg is always laid before the next one comes and replaces it. The layings follow one another slowly. At a temperature of 20° C., the maximum of eggs laid in twenty-four hours is of 12 to 13.

¹ Die..... Nematoden der niederländischen Fauna, 1884, p. 94, pl. XIII, fig. 54.

The maturation of the eggs is interesting to follow. When the ovum is ripe and ready to pass into the uterus, it displays a beautiful, clear germinal vesicle of a diameter of 8 to 9 μ , with a large opaque and spherical nucleolus of a diameter of 4 μ . Slightly before entering the narrow passage of the oviduct (tuba), the germinal vesicle rapidly loses its regular contour, probably through the dissolution of its peripheral membrane. It stretches along its length and becomes much less visible. The ovum then begins to enter the oviduct, across which it stretches as in a screwing die; the passage lasts about two minutes. Once it has arrived into the uterus, it occupies the entire anterior region including the dead end that first opened to receive it. The deformed and stretched germinal vesicle is found along the edge and in the posterior third of the new uterine egg. This egg first only occupies the anterior part of the uterus; but it does not take long for it to go down slowly towards the posterior part, into which it arrives and remains after 8 to 10 minutes of slow sliding.

It resides in this state in the uterus for two hours (temperature 18° C.), secretes there its chitinous shell, and then is laid. At this time, the vitellus almost completely fills the entire cavity of the shell. Immediately after the laying starts also the expulsion of the polar body. It takes place at the bottom of a deep indentation that is formed by retraction of the vitellus at the level of the first third of the egg that came out at laying; said otherwise, where we saw the deformed germinal vesicle come and reside at the beginning. This development lasts approximately an hour, during which the nucleus stayed invisible. Then the vitellus shrinks towards the middle region of the shell, leaving quite large empty spaces at the extremities. At the same time, the nucleus reappears as a beautiful clear vesicle, which is spherical, nucleolated and of dimensions almost equal to

those of the primitive germinal vesicle. At this time, the egg is ready for the first blastomere division, which takes place approximately one hour later, or two hours after laying.

I have minutely followed this entire development on several eggs and I could never see the two clear spots of the male and female pronuclei come close and mate, as they are easily seen in hermaphroditic and dioic species. This observation is important to help demonstrate parthenogenesis in this *Cephalobus*.

Its development and growth are very slow; however, its lifespan is long. At a temperature of 20° C., the eggs take three days for their embryogenesis until hatching. At the same temperature, the larvae require 10 to 11 days to grow until the laying of their first egg. Here is actually the complete history of an individual that I followed day after day from the time of its hatching until its death of old age. Having come out of the egg on October 24, it laid its first egg on November 3. The layings then continued regularly until January 4 with ups and downs, caused by the variations in temperature and food quality. But from January 4 on, they slowed down considerably, giving only one or two eggs per day or even sometimes none at all. This state went on until the end of January, when the layings of fertile eggs were replaced by sterile eggs, aborting and falling apart right after they were laid. The production of these unfertilized eggs continued during the whole of February; then, in the first days of March, the animal having lost the energy that is necessary for the expulsion of these unfertilized eggs, some of them accumulated in the uterus, where they fell apart. The animal further lived until March 28, becoming almost absolutely inert, and it perished of senile exhaustion. In summary, it had lived for five full months and laid a total of 415 fertile eggs.

In the cultures of this species, several hundred individuals passed under my eyes, among which I never encountered a single male. Moreover, I examined the genital organ of many adult females with the greatest care and with the best objectives, without ever catching sight of the least trace of spermatozoa. Given the simplicity of these organs, they are easy to explore in all their parts and if spermatozoa were stored in one of their folds, they would not have escaped my investigations. *Cephalobus dubius* is thus very certainly a parthenogenetic species.

When describing the mouth and the tail, I insisted on the differences in the conformation of these organs that distinguish two varieties or races, which I baptized race *apicata* and race *rotundata*.

In all other parts of this organism, the two races are absolutely identical to each other, and when one studies them for a long time as I did, no doubt can remain about the unity of the species. Considered for themselves, the differences between them could therefore appear of mediocre significance. But this significance becomes serious once one knows that these races are permanent and live isolated and independent of each other.

Among the individuals that I studied, all those coming from Algiers, Taouessera, Haci-Morra and Antananarivo belonged to the *apicata* race; whereas all those from Aïn-Sfissifa, Jurjura, Bousaada and Chott-Chergu had the *rotundata* form. We therefore have here two varieties, which, in the locations where they are found, reproduce and are perpetuated, each keeping intact its particular conformation. I made numerous and long cultures of both types. Their conformation was maintained from one generation to the next without modification and without letting the least hint of a transition from one variety to the other. Their particular characters

are thus absolutely fixed and permanent. Since the parthenogenetic state of the two varieties prevents any cross between them, one cannot see any reason why this fixity of characters could be shaken, allowing the two forms to merge and return to a common type.

This fact has a high significance. It shows us, indeed, in a form with a purely parthenogenetic reproduction, the formation of races (species?) that are distinct and fixed in the state of nature. If, as is perhaps possible, one would demonstrate that *Cephalobus nanus* of De Man is itself a third form of the same type, which was fixed and became hereditary, the significance of these observations would be further increased. It has indeed been claimed that the mixing of the *ancestral plasmas* through amphimixis was absolutely necessary for the creation and fixation of new specific characters. According to this theory, any possibility of permanent and hereditary variation would be denied to those beings with purely parthenogenetic reproduction. The facts that we just described stand in absolute contradiction with this viewpoint.

CEPHALOBUS LENTUS mihi

I found this species only in sandy soil accumulated under a clump of *Anabasis aretoïdes* that was brought back by Mr. Flamand from the Feidja of Djenien Bou Rezg (South-Oran region).

Measurements:

| | ♀ | YOUNG at hatching |
|---------------------|---------|----------------------|
| Body | 905 □ | - |
| Esophagus | 211=¹/₄ | 97=¹/₂₆ |
| Tail | 46=¹/₁₉ | 18=¹/₁₄ |
| Vulva | 600 | |
| Diameter | 46=¹/₁₉ | 16=¹/₁₆ |
| Buccal cavity | 14=¹/₁₅ | 6=¹/₁₆ |

The body (pl. XXIV, fig. 3), quite regularly cylindrical and relatively thin, only thins out in the front in the esophagus region, ending with the buccal truncation. In the rear, it rounds up suddenly at a short distance from the anus. Its general aspect is rather opaque in well-fed individuals, because of the presence of many albumino-fatty granulations in the wall of the intestine and in the connective tissue.

The cuticle is clearly transversally striated. The lateral membrane is barely marked and it can only be distinguished on emaciated individuals. It appears under the aspect of a narrow band (pl. XXIV, fig. 6 *m*), bordered by two fine protruding lines and divided longitudinally in two by a third line, parallel to the two others.

The mouth (pl. XXIV, fig. 4) is lined by six pointed lips, separated by large concave serrations; the back of these teeth protrudes slightly outwards. They are as strongly chitinized as the rest of the body surface. Within the circle that they form, are found three bifurcated chitinous appendages that greatly protrude in the front. Analogous appendages, but half as long, have already been described in *Cephalobus ciliatus*¹. The buccal cavity is long and narrow and presents the usual chitinous thickenings of *Cephalobus*. Its length equals 1/15 of the total length of the esophagus, as measured from the anterior extremity of the body.

The esophagus (pl. XXIV, fig 5) has no anterior bulb-shaped swelling. Its anterior part is a little more than twice as long as its posterior part (neck and bulb). The neck is quite narrow. The bulb is a fourth longer than wide: its valves (teeth) are very strong. In the front, the esophagus forms a thick sheath that envelops the buccal cavity until its anterior end.

¹ See DE MAN: *Die frei... Nematoden der niederländischen Fauna*, 1884, p. 100, pl. XV, fig. 61.

The tail (pl. XXIV, fig. 6) is short; its free extremity is thick and rounded, and still almost half as wide as its origin. Towards the middle of its length, it bears a pair of lateral papillae, which are extremely fine and difficult to see. One can only succeed in seeing them on individuals that lie on the ventral side and are rather strongly compressed.

In well-fed individuals, the cells of the intestine are filled with albumino-fatty granulations, which accumulate as storage substances. These granulations also invade all the connective tissue in the walls along the esophagus and inside the tail. They give a general opaque aspect to the body. Birefringent corpuscles are never found.

The nerve ring (pl. XXIV, fig. 5 c), of fibrous structure, is seen with some difficulty. It envelops the esophagus neck at its anterior extremity and bends obliquely towards the ventral side, extending out towards the excretory pore.

The excretory apparatus (pl. XXIV, fig. 5 p) is difficult to observe. The small unpaired chitinized tube can be seen relatively easily. It first describes a narrow and long loop directed towards the front, then folds back towards the bulb. The pore is located slightly in front of the bulb. The lateral canals can only be observed on emaciated and strongly compressed individuals. I did not succeed in following them beyond the vulva. A unicellular gland is found close to and behind the pore.

The vulva (pl. XXIV, fig. 3 v) is located at the boundary between the second and third thirds of the total body length. Its lips are slightly swollen. The vagina is very short. The genital tube is unpaired, with a relatively long uterus. Taking the shape of a narrow sac, it extends directly in front of the vulva and finishes in a dead

end. It never contains more than one egg, always laid before the arrival of the next. Behind the vulva is found an aborted genital tube in shape of a narrow transparent sac, of a length equal to two and a half times the body width. The uterus connects to the ovary through a narrow oviduct inserted at the end of the second third of its length. The ovary comes back down directly along the body and ends slightly in front of the extremity of the intestine. In the middle, between this extremity and the vulva, it always forms a rather long double loop.

The eggs (pl. XXIV, fig. 7), of oblong shape rounded at the extremities, measure 66 \square in length and 26 in width. Their chitinous shell is shagreened (covered by small protuberances). The vitellus is colored in dark mahogany brown.

The multiplication of this *Cephalobus* takes place very slowly. The layings occur at a great interval from each other, with a maximum of 8 to 9 per 24 hours, at a temperature of 26 to 27° C. Only a single egg can ever be seen in the uterus. These eggs are always laid before their first cleavage. The species is thus essentially oviparous.

The development and the growth are very slow; however its lifespan is quite long. At a temperature of 26 to 27° C., the eggs take 4 days 1/4 to develop from laying to hatching and the larvae 16 days to reach a perfect adult state and lay their first egg. Here is actually the complete history of a female, which I followed day after day from its origin until its death of old age. It came from an egg that was laid on July 29 and hatched on August 2 in the evening. It grew until August 17, when it did its first laying. The layings continued until October 5, and from this day, it lived fully sterile to perish of senile exhaustion on November 14. During the fertile period of 49 days, the daily number of eggs was quite variable and never exceeded 9. The

total of the layings was of 315 eggs. This female had lived for a total of 105 days.

The juveniles coming out of the egg (pl. XXIV, fig. 8) are all born with their complete organs, except for the genital apparatus which is still in an extremely rudimentary state. But the proportions of the parts of the body are very different from those of the adult state. Indeed, the esophagus and the tail equal $1/2.6$ and $1/16$ of the total length, respectively, instead of $1/4$ and $1/19$. It results thereof that, in the final growth, the greatest part is contributed by the middle region of the body, which multiplies 4.5-fold, whereas the esophagus and the tail only multiply 2 and 2.5-fold.

This species suffers long dessications with impunity and revives as soon as it is moistened again. I have seen some regain life in a few hours, which were dessicated for five years. In my cultures, I fed it with diluted rotten flesh.

The movements are slow and heavy.

Cephalobus latus is a species with a parthenogenetic reproduction. I made sure of it:

- 1° By ascertaining the absolute absence of males;
- 2° By rearing juveniles and following them until the adult state; all became females, which laid eggs that developed regularly;
- 3° By examining at high magnification the genital organs of the adult females, and not ever seeing the least trace of spermatozoa;
- 4° By observing, in an immobilized individual, the arrival of an egg into the uterus and following it after its laying until the first division into two blastomeres. I repeated this observation several times, and during its whole length never saw two distinct pronuclei, nor their coming together and their mating, phenomena that are so easy to follow in the dioic or hermaphroditic species.

PLECTUS CIRRATUS Bast.

BASTIAN. — *Monograph on the Anguillulidae*, 1865, p. 119, pl. X, fig. 81, 82.

DE MAN. — *Die frei..... Nematoden der niederländischen Fauna*, 1884, p. 110, pl. XVII, fig. 68.

I found this species twice in Algeria; a first time in humus that was sampled by M. Ficheur, at an altitude of 1,500 meters, on the Southern slope of the Jurjura; a second time in the meadows of Maison-Carrée, close to Algiers. I cultured it on depression slides, by feeding it with diluted rotten flesh.

The drawings and the description of De Man being very sufficient, I will satisfy myself with presenting the observations that I could gather on the biology and sexuality of this nematode.

Growth and multiplication are not very rapid. At a temperature of 26° C., the eggs take 44 hours to undergo their embryogenesis until hatching. At the same temperature, the larvae require 10 days to grow until the adult state and the laying of their first egg. Still at the same temperature, it lays a maximum of 30 eggs per day. I did not make any observations on the total number of its eggs, nor on its lifespan.

This species is essentially oviparous. It lays its eggs most usually before the first cleavage. Therefore no more than 5 to 6 eggs can ever be seen together in each uterus.

The maturation of its eggs is interesting to follow. When the ova of the anterior region of the ovary are mature and ready to enter the oviduct to pass into the uterus, they display a very conspicuous and large germinal vesicle, of slightly elongated shape, measuring 12 to

13 μ , and endowed with a large spherical nucleolus, measuring 5 to 6 μ . They cross the narrow strait of the oviduct by stretching and fall into the uterus, where they curl back on themselves and take a slightly irregular shape, but close to the spherical state.

At the time of passage in the oviduct (tuba), the germinal vesicle is still intact, with the abovementioned dimensions and thus still very conspicuous. But as soon as the ovum has entered the uterus, the vesicle undergoes a deep change that renders it almost invisible. On some eggs where it is most likely located at the bottom, it can be searched in vain. On the others, where it is located on top, one can recognize it under the aspect of a diffuse spot, of circular shape and half as big as the primitive vesicle.

The eggs stay in this state in the uterus for half an hour to three quarters of an hour (temperature 27 ° C.) and wrap themselves in their shell, which is covered with small spikes.

I examined at high magnification a great number of these uterine eggs, and always, without a single exception, I saw them having a single nuclear spot. This observation is important for the demonstration of their parthenogenetic state. Indeed, if this nematode had been hermaphroditic and its eggs had been fertilized, I should certainly have come across one of these eggs with two yet unfused pronuclei, as can be easily seen in the dioic or hermaphroditic species.

After about three quarters of an hour in the uterus, the eggs are laid with a well-formed shell. At the very moment of their laying, the vitellus fills out the whole cavity of the shell without letting any empty space. At the same time, the nucleus or germinal vesicle has become completely invisible. It is probable that this invisibility is

caused by the labor of production of the polar body, which I actually never could see in any egg.

This invisibility lasts for approximately one hour to one and a half hour; then the nucleus reappears under the form of a beautiful clear nucleolated vesicle, of the same dimension as the primitive germinal vesicle.

At the same time, the vitellus retracts, letting rather large empty spaces in the cavity of the shell, and is agitated by amoeboid movements, which are clearly visible with the eye and which drag the nucleus sometimes on one side, sometimes on the other. This state lasts approximately for one and a half hour, then comes the cleavage into two blastomeres. The two first blastomeres are still endowed with amoeboid movements; but I did not observe any at the stage of four blastomeres, which occurs approximately one hour after the first division (temperature 27° C.).

This maturation and this laying are perfectly identical to that observed and described in the *Cephalobus dubius*.

Above, we stated the parthenogenetic state of this *Plectus* by basing us on the mode of maturation of its eggs. To this demonstration, we can add the followings:

I observed many individuals, without ever encountering a male. I reared juveniles that had been isolated since the time of their hatching, and all became females that laid regularly developing eggs. These fertile females could have been hermaphrodites.

I had more difficulties to ascertain directly the absence of hermaphroditism. The organ of reproduction is rather difficult to see in its entirety; especially in the upper region of the uterus, where it narrows into an oviduct (tuba), which folds back underneath and inside to connect to the anterior part of the ovary. But by

appropriately compressing live individuals, I could ascertain many times the complete absence of spermatozoa in the entirety of the uteri and oviducts of females that were laying regularly developing eggs.

I moreover took young females reaching the adult age, but without yet a single egg in the uterus. At this time, in the hermaphroditic species, one can witness the formation of sperm in the upper region of the ovary and its storage in a receptacle. The last germinal cells in this ovary region take the shape and development of spermatoblasts, which are always smaller than the mature ova ready to enter the uterus. In the *Plectus*, I never saw spermatoblasts, but always ova.

Finally, by cutting adult females in two with a fine blade, either in pure water, or in 1 % acetic acid, one can almost always obtain the hernia of an ovary and of its uterus, which then become easy to observe in their entirety. I did not see therein any trace of a seminal receptacle nor of sperm.

I brought the greatest care to these verifications, because of an anatomical particularity of the oviduct, which troubled me for a long time by making me believe in the possibility of hermaphroditism. The uterus of this *Plectus* and the oviduct are composed, like the intestine, of small polygonal cells without apparent nucleus. In the region where the oviduct and the uterus meet, one or two of these cells often contain small opaque corpuscles (pl. XXV, fig. 1 c c), of spherical shape, that simulate rather well spermatozoa of very small size. But, as I said above, I could never see the spermatoblasts of these pseudo-spermatozoa. Moreover, their number was very insufficient to account for the fertilization of the many laid eggs of each female. Finally, I often searched for them in vain in young

females that had already their first egg in the uterus. These corpuscles cannot have anything in common with spermatozoa.

De Man states the existence of males and gives their description. He adds that they are extremely rare, whereas the females are very common. Bastian, like myself, has not seen any male. If the observation of De Man is exact, we would have in this *Plectus* a parthenogenetic species, in which the male sex would sometimes reappear as an atavistic sign of an ancient dioic state.

APHELENCHUS AGRICOLA DE MAN

BÜTSCHLI. – (*Aphelenchus avenae*). *Beiträge*, etc. 1873, p. 46, pl. III, fig. 15.

DE MAN. – *Die Nematode*, etc., 1884, p. 138, pl. XXI, fig. 90.

I found this species twice in Algeria, the first time in soil coming from the region of Bousaada, the second time in soil sampled on the ridge of the forest of Teniet-el-Hâd. Bütschli encountered it in Germany and de Man in the dunes of Holland. It lives in light, more or less sandy soils, among plant roots.

Measurements:

| | | |
|---------------------|----------|---------|
| Body | 858 □ | 1015 □ |
| Esophagus | 98=¹/₈.₇ | 105=¹/₉ |
| Tail | 29=¹/₂₉ | 29=¹/₃₅ |
| Vulva | 657 | 786 |
| Diameter | 30=¹/₂₈ | 46=¹/₂₂ |
| Buccal cavity | 20=¹/₅ | 18=¹/₆ |

The rather thin body (pl. XXV, fig. 2) of uniform diameter tapers slightly and gradually in the esophagus region to end by the truncation of the mouth; its posterior extremity is rounded. Its general aspect in well-fed individuals is rather opaque; opacity that

is caused by the many granulations stored in the walls of the intestine.

The cuticle is finely transversally striated. The lateral membranes have the shape of two bands of a width larger than the body diameter by more than one third. These bands bear 10 to 12 fine longitudinal striations (pl. XXV, fig. 5 *m*). The transversal striation stops right at the edge of these bands. They maintain their width from the extremity of the tail until beyond the esophagus bulb, then narrow rapidly and end by completely disappearing in the buccal region. De Man described these striated bands very well; but he confuses them wrongly with the lateral fields that are found below and which belong to the muscular-cutaneous layer and not to the cuticle.

The mouth (pl. XXV, fig. 3) ends the anterior extremity without any special change that would separate it from the rest of the body. The cuticle is only thinner and without striations in a small zone. There are no lips, nor papillae. The stylet of the buccal cavity is clearly tubular and its walls continue without discontinuity with the chitinous tube of the esophagus. There is simply a slight thickening at their junction point. Another much smaller thickening is also found in the middle of its length.

The esophagus (pl. XXV, fig. 4) presents the usual conformation for *Aphelenchus*. The bulb is powerful and with a clearly fibrous structure. It always contains two to three finely granular masses, of glandular appearance. Its internal chitinous wall is strongly thickened. The part of the alimentary canal that follows, the substance of which is always clearer and without granulations, represents for me the second part of the esophagus, as known in the neighboring genus *Tylenchus*. In most *Aphelenchus*, this clear part

extends without discontinuity with the intestine proper. Therefore it has generally been related with this organ. But, in *Aphelenchus agricola*, it is separated from it by a thin, barely visible narrowing (pl. XXV, fig. 4 e) and even ends with a very small bulge. Moreover, the central chitinous canal is narrow and so to say closed along its whole length, whereas it opens and widens immediately after the slight narrowing, thus marking the true beginning of the intestine. Let us further add that the nerve ring and the excretory pore are connected with this second part, as occurs in *Tylenchus*, *Rhabditis* and all other nematodes with an esophagus with two bulbs. All these facts clearly prove that Bütschli was right (*Beiträge*, p. 45) when he expressed the thought that the clear anterior part of the intestine of *Aphelenchus* could well represent the second part of their esophagus. All this part is enveloped by a glandular tissue, which extends even further behind and contains some voluminous and clear nuclei.

The tail (pl. XXV, fig. 5) is very short and barely longer than the rectum. It does not thin out much and becomes suddenly rounded.

The intestine proper first starts in the front being rather narrow (pl. XXV, fig. 4 i). In this narrow part, its walls are always transparent and contain very few granulations. But as it thickens, its walls keep large quantities of storage substances in well-fed individuals, in the form of albumino-fatty granulations. Birefringent corpuscles can never be found. These granulations give the intestine its rather great opacity. The rectum has at the level of its junction point with the intestine a length equal to the body width. I did not observe any rectal glands.

The nerve ring (pl. XXV, fig. 4 c), of fibrous structure, is not conspicuous and is somewhat difficult to see. It is located very close

and behind the large bulb and bends slightly obliquely towards the ventral side.

The excretory organ (pl. XXV, fig. 4 *p*) must be observed on emaciated and appropriately compressed individuals. The pore is located slightly behind the nerve ring. The chitinized unpaired canal is not very conspicuous. I nonetheless could follow the longitudinal canal rather far in the posterior direction.

The vulva (pl. XXV, fig. 2 and 6 *v*) is located slightly behind the last quarter of the total body length. Its lips protrude slightly. The vagina is relatively long and extends perpendicularly to the body axis. The uterus has a length equal to three times the body width. Its walls are somewhat irregular, thick, and composed of many hyaline and transparent cells. In the front, it widens and becomes linked to the ovary, to which it connects without an intermediate oviduct narrowing like a tuba. The ovary extends upwards directly along the intestine, without forming a curve nor a loop and ends quite far behind the esophagus.

The eggs (pl. XXV, fig. 7) of cylindrical shape, rounded at the extremities, measure 79 \square in length and 23 \square in width. Their shell is thin and smooth.

Aphelenchus agricola is essentially oviparous. The eggs are always laid before their first cleavage and no more than one can be seen at any time in the uterus. The layings follow each other slowly and do not much exceed the figures of 20 to 25 eggs per day, at a temperature of 24° C. At the same temperature, these eggs take two and a half days to undergo their embryogenesis until hatching, a duration that indicates a very slow development and growth. But I could not ascertain it directly, this species not being sustainable with my methods of culture.

This *Aphelenchus* is obviously endowed with a great faculty for reviving. The soil of Bousaada in which I found it was completely dessicated when I received it. I organized with it a terrarium, by moistening it and, several days later, I discovered this nematode vigorous and healthy on the small pieces of flesh that I had deposited at the surface of the wet soil.

Aphelenchus agricola is a parthenogenetic species. I convinced myself of it by the following observations:

1° Neither Bütschli, nor De Man, nor myself have ever encountered a male.

2° Chance having put under my hand a young female undergoing its fourth and last molt, I isolated it and could feed it for three days. It laid under my eyes a few eggs, which all developed regularly.

3° I examined at high magnification the uterus of several females that were laying good eggs and in none of them could I see any trace of spermatozoa. Yet the uterus of this species is very transparent and easy to explore on slightly compressed individuals.

These proofs are more than sufficient to demonstrate parthenogenesis in this species.

ALAIMUS THAMUGADI mihi

I found this species only once. I had brought it back in sandy soil that was attached to the shoots and roots of a moss tuft sampled in the ruins of the Byzantine fortress of Timgad, province of Constantine.

Measurements:

| | |
|-----------------|--------------|
| | ♀ |
| Body | 1144 □ |
| Esophagus | $214=^{1/5}$ |
| Tail | $64=^{1/18}$ |

| | |
|----------------|--------------|
| Vulva | 457 |
| Diameter | $25^{=1/41}$ |

The body (pl. XXVI, fig. 1), thin and slender, tapers slightly and gradually towards both ends. In the front, it is truncated by the mouth, in the rear, it ends in a conical tip, slightly obtuse and curved towards the ventral side. Its general color is of a rather light grey.

The cuticle is colorless, smooth and without transversal striation, nor lateral membranes. In ten individuals that I had left for 48 hours to macerate in a solution of 1 % acetic acid, it remained simple and did not split into two, as always occurs in such circumstance in other nematodes. It is thus formed of a single layer only.

The buccal extremity (pl. XXV, fig. 8) rounds up regularly, with its small opening in the center. No differentiation distinguishes it from the rest of the body. I looked with the greatest care for the lateral organs that de Man describes in the two species that he described and could not see the least trace of them. There are no lips, nor papillae.

The esophagus (pl. XXVI, fig. 2) is regularly thin and narrow along all its length, except at its posterior extremity where it suddenly bulges, to form a well-outlined bulb, twice as long as wide. A nucleolated, large and clear nucleus can always be seen within the amorphous substance of this bulb.

The tail (pl. XXV, fig. 9) has the shape of an elongated cone, with a slightly obtuse tip, curved towards the ventral side.

The intestine does not contain many granulations and its general aspect remains light greyish. At its junction point with the rectum, it is endowed with a narrow diverticulum (pl. XXV, fig. 9), directed posteriorly and of a length equal to half of that of the tail. The

rectum is short; its length only equals half of the width of the body at its level.

The nerve ring (pl. XXVI, fig. 2 c), of fibrous structure, is located slightly behind the middle of the esophagus. It envelops it by bending obliquely towards the ventral face. – There are no excretory organ nor pore.

The vulva (pl. XXVI, fig. 1 v), without protruding lips, is located behind the bulb at a distance equal to the esophagus length. The vagina is very short. The unpaired genital tube extends behind the vulva. It is very little developed and its total length is notably shorter than half of the distance that separates the vulva from the anus. Considering the fineness of its parts, its structure is rather difficult to untangle. Here is however how I understood it. First, starting from the vulva, the relatively long uterus, usually containing two eggs finishing their maturation and secreting their shell. Then a long and thin oviduct, which I was not able to see, but that would extend towards the posterior direction and form the terminal bend by connecting with the ovary, itself directed in the opposite direction towards the vulva. The ovary is very little developed, has a length slightly shorter than the uterus, at the anterior level of which it ends.

The eggs (pl. XXVI, fig. 3), of elliptical shape, rounded at the extremities, measure 74 \square in length and 30 \square in width. Their shell is colorless, thin and smooth.

The movements of this *Alaimus* are extremely slow and heavy. The animal twists and contorts slowly, almost without moving along.

This new *Alaimus* distinguishes itself from the two species known so far by its proportionally much shorter tail, by the absence of lateral organs, by the well-outlined esophagus bulb, by the vulva

position and by some other features on which it is unnecessary to insist.

The multiplication and development must be extremely slow. I do not have rigorously precise observations on the succession of the layings; but from what I could perceive, this animal only lays an egg every two or three days, at a temperature of 20° C. These eggs are always laid before the first blastomere division. At a temperature of 20° C., they require 14 to 15 days to undergo their embryogenesis until hatching.

Alaimus Thamugadi is parthenogenetic. I ascertained it by minutely examining 30 encountered individuals, which were all females. I looked with the greatest care and the highest magnifications for the presence of spermatozoa in the genital organ of these females, without seeing the least trace thereof. These females however all had laid eggs that were developing regularly. Consequently, not finding males nor spermatozoa, one necessarily had to admit parthenogenesis for these eggs.

MACROLAIMUS CRUCIS n. gn. n. sp.

I have encountered this species on two different occasions; the first time in sandy soil stuck to a tuft of *Anabasis aretoïdes* brought back by Captain Lacroix from the wadi Zoubia (South-Oran region); the second time in soil mixed with a tuft of grass sampled close to Biskra. I succeeded in having some individuals living on a depression slide, feeding them with diluted rotten flesh. I dedicate it to Captain Lacroix.

Measurements:

| | |
|-----------------|------------|
| Body | ♀ 958 □ |
| Esophagus | 200=¹/₄.₈ |

| | |
|---------------------|---------------|
| Tail | $60=^1/_{15}$ |
| Vulva | 557 |
| Diameter | $42=^1/_{22}$ |
| Buccal cavity | $10=^1/_{10}$ |

The body (pl. XXVI, fig. 4), slender and thin, goes on tapering towards both ends. In the front, it ends by the mouth truncation; in the rear, it thins out rapidly in a fine conical tip, always bent slightly towards the ventral side. In well-fed individuals, it has a rather opaque general aspect, which is caused by the granulations of the intestine.

The cuticle, on living specimens, appears smooth and without striation nor ornament of any sort. But when it has macerated in 1 % acetic acid for an hour, one can finally see a very fine transversal striation.

The mouth (pl. XXVI, fig. 2) is widely open at the anterior end, and does not show neither lobes nor lips on its circumference. On its immediate rim are inserted six short and thickset bristles, which, by certain aspects, could sometimes make one believe in the existence of six little-protruding lips, of which they would represent the papillae. The buccal cavity, in the shape of a wide cupule, is of one third deeper than wide. Its wall is divided into two distinct halves, one anterior, thin and transparent, one posterior, thick and strongly chitinized, of blackish aspect. This conformation of the buccal cavity is characteristic of the new genus and reminds one of that of some *Diplogaster*. This resemblance seems even more complete in some individuals that show a sort of tooth (pl. XXVI, fig. 6) inserted in the middle of the buccal wall. But the presence of this tooth is not constant and it even seemed to me to be more often absent than present.

The esophagus (pl. XXVI, fig. 7) completely resembles by its conformation that of *Cephalobus*. Its anterior part, rather thick, does not form any bulge. The narrow neck is rather long and, with the bulb, almost equals in length the anterior part. The bulb, well-developed but somewhat narrow, is one and a half times longer than wide. All these parts have a clearly fibrillar structure. The internal chitinous coating of the anterior part always describes slight sinuosities. The valves (teeth) of the bulb are vigorous and appeared to me to resemble those of *Rhabditis* rather than those of *Cephalobus*.

The tail (pl. XXVI, fig. 8), not very long and of conical shape, ends in a fine tip. It is always slightly twisted towards the ventral side. I did not see any lateral papillae.

The intestine, in well-fed individuals, always contains within its walls many granulations of storage substances. These granulations give it its greyish, opaque, aspect. There are never any birefringent corpuscles. – The rectum has a length equal to the body width at the level of the end of the intestine. It is endowed with rather small glands at its junction point with the latter. The anus has a bulged and protruding posterior lip.

The nerve ring (pl. XXVI, fig. 7 c), of fibrous structure, envelops the narrow neck of the esophagus at the extremity of its anterior third. It is bent very obliquely towards the ventral side.

The pore of the excretory organ (pl. XXVI, fig. 7 p) is located slightly in front of the bulb. The unpaired canal is very short and in a direction vertical to the body axis. It branches immediately and gives rise to two lateral canals directed towards the rear. I did not see branches extending towards the front, and I am convinced that they do not exist. After the branching, each of the lateral canals

immediately crosses, with a very sinuous course, a thick and long gland, endowed with one or two nuclei; then they continue towards the back, always describing many sinuosities. I succeeded in following them until the level of the vulva, beyond which they disappear so completely that I am ready to believe that they do not exist any more.

The unpaired genital organ (pl. XXVI, fig. 4 and 9 *v*) is conformed according to the type of *Cephalobus*. The vulva has highly protruding lips, the anterior one being slightly thicker than the posterior one. The vagina is very short and runs directly along the body axis. The genital tube is relatively little developed. Its anterior bend stops behind the esophagus, at a distance almost equal to the length of this organ. Its blind posterior extremity ends in front of the extremity of the intestine at a distance equal to two widths of the body.

The uterus (pl. XXVI, fig. 9 *u*) has an oblong spindle shape; it continues with the oviduct (*o*), in which it passes through a gradual narrowing, without clear boundary between the two. The oviduct extends until the anterior bend of the genital tube. These two organs are composed of many small polygonal cells, as in the *Plectus*. The ovary folds back towards the rear, with a straight course, without folds nor loops.

The eggs (pl. XXVI, fig. 10), of elongated shape, rounded at the extremities, measure 80 \square in length and 26 in width. Their shell is thin and smooth.

This nematode is endowed with very lively movements. Not that its circulates and moves around much; but it constantly wriggles and contorts itself, describing the most varied loops. Especially, all the esophagus region is endowed with an extreme motility and twists in

all directions with great agility. This motility reminds that of the ciliated infusoria of the *Lacrymaria* genus. It is very characteristic for the species, which is immediately recognized by these movements, as soon as one has observed them once.

This new genus belongs to the family of the Rhabditids. By its mouth and its buccal cavity, it is connected with *Diplogaster*. Its esophagus and genital apparatus remind those of *Cephalobus*. The conformation of its tail is also frequent in the latter genus.

The multiplication of *Macrolaimus crucis* is very slow. Even at a temperature of 26° to 27° C., it only lays two to three eggs per twenty-four hours. Only a single egg can ever be seen at a given time in the uterus. The eggs are always laid before their first cleavage, as I actually always observed in all other parthenogenetic species studied so far. It thus results that, although rather common, this animal is only encountered a few individuals at a time.

Its development and growth are also very slow. At a temperature of 26° to 27° C., the eggs take fifty hours to undergo their embryogenesis until hatching. At the same temperature, the larvae require 10 to 11 days to reach the adult state, until their first egg-laying. I do not have any observation on their total lifespan.

This nematode reproduces through parthenogenesis. I ascertained it:

- 1° By observing a complete absence of males;
- 2° By raising juveniles, isolated since the egg until the adult age and seeing them lay eggs developing regularly;
- 3° By examining at the highest magnification about ten adult females, without being able to see in their uterus the least trace of spermatozoa.

SUMMARY AND CONCLUSIONS

I. - In the previous pages, we studied 18 species of nematodes that reproduce without the help of distinct males. Among these 18 species, 2 of them, *Rhabditis dolichura* and *R. Schneideri*, were previously known to lack males. We thus demonstrated their absence in 16 further species. Among these 16 species, 13 are entirely new; the 3 others had been described before, but their true sexual state had remained unknown.

We saw in the historical introduction at the beginning of this work (p. 9) that hermaphroditism and parthenogenesis of 18 other nematodes were already known through different observers. We gave the list of these species. This list, together with the 16 new species that we studied, brings to 34 the present number of nematodes that clearly reproduce without males. Among those 34 species, 25 are hermaphroditic and 9 are parthenogenetic.

These figures are far from being final and are surely destined to grow in the future. Hermaphroditism and parthenogenesis are very widespread in nematodes. Suffice us to recall that we showed in the historical introduction (p. 11) that, among the 206 species of freshwater and terrestrial nematodes that were described by Bastian, Bütschli and De Man, 85 were only known through the females. Thus, there lies a rich mine of new observations and discoveries to be made. I shall add that, according to my personal experience, the completely undescribed species are very likely to be even more abundant.

These 34 unisexual nematodes are distributed among the 12 following genera: *Rhabditis*, *Diplogaster*, *Cephalobus*, *Plectus*, *Allantonema*, *Bradytrema*, *Macrolaimus*, *Angiostomum*,

Strongyloides, *Dorylaimus*, *Aphelenchus* and *Alaimus*. The representatives of four of these genera are parasitic species. All others are free-living.

The *Rhabditis* genus alone provides almost half of this share (46 species); simply, we believe, because its representatives, lending themselves without difficulties to methodical cultures, are easier to study, and also because this genus being endowed with a great variability contains a large number of species. We therefore do not think that unisexuality is particularly frequent in this genus compared to others. The *Plectus* and *Dorylaimus* genera, having many representatives, will certainly bring many recruits to hermaphroditism and parthenogenesis, once all their species are suitably studied.

II. - By their general morphology and biology, these unisexual nematodes cannot be distinguished at all from their congeners with two separated sexes. They all appear with the general aspect and the ordinary conformation of females. They can only be distinguished from neighboring species by ordinary specific nuances, and their particular ovogenetic system can only be recognized through the minute study of their genital apparatus and above all of its functioning. Hermaphroditism and parthenogenesis did not affect in any particular manner the general and specific characters of these beings. Only their genital apparatus has been modified: even then, these alterations concern much more the functioning than the layout and structure of this apparatus.

Three only of these nematodes are ovo-viviparous, *Rhabditis elegans*, *R. caussaneli* and *Diplogaster robustus*. All others, essentially oviparous, lay their eggs either before any blastomere

divisions, or after only the three or four first divisions. But this results from the poor development of the uterus of these species and is not obviously related to their ovogenetic system.

All these types with unisexual generations are only represented, as we reported, by individuals corresponding to the feminine form of the species. These females themselves do not differ in anything peculiar from their congeners with separate sexes. They distinguish themselves only by the functioning of their genital apparatus. The latter, in its structure and general conformation, is absolutely identical to that of other species. We are thus entitled to state that parthenogenesis and hermaphroditism, when they developed in these nematodes, only exerted a modifying influence on the products of the genital apparatus. The rest of the organism remained absolutely invariable.

In the parthenogenetic species, the ova underwent the special development, which is still ill-known, that allows them to mature and develop without fertilization.

III. - In the hermaphroditic females, as we ascertained it for each of the described species, the genital apparatus, when approaching maturity, first starts to function as a testis and produces a certain quantity of sperm. At this time, the young germinal cells of the anterior ovary region, which had started to grow, can be seen (pl. XXI, fig. 7 A) to arrest their growth, divide twice and become small spermatozoa. These are kept and stored in an appendix of the uterus, which plays the role of a seminal receptacle. Later, with the animal developing further and the genital organ maturing (pl. XXI, fig. 7 B), the germinal cells of the anterior ovary region continue to grow, accumulate a large amount of nutritive vitellus in their

cytoplasm and transform themselves into large ova, ready for fertilization.

The latter takes place as soon as these mature ova detach from the ovary and move into the uterus. They are indeed compelled, in order to undergo this movement, to cross the seminal receptacle, where the spermatozoa that were formed before them have been stored. At this time, one of the spermatozoa comes in contact with each of them, sinks into its cytoplasm and joins and fuses its pronucleus to the ovular pronucleus.

These nematodes are thus protandrous hermaphrodites with autogamous fertilization. Their fertilization always takes place with the strictest inbreeding, since ova and spermatozoa derive from a single genital gland and are therefore fraternal products, in the most rigorous and physiological meaning of the word. These hermaphroditic females do not possess any arrangement, any apparatus that would allow them to mate to each other and cross-fertilize. The females lacking in addition active males that would be able to mate, the gonadal elements produced by their genital apparatus are necessarily and fatally condemned to autogamous fertilization. They are thus forbidden any sort of cross-fertilization. We shall return later to this question.

This protandrous hemaphroditism, with successive maturation of the genital elements, the male elements always preceding the female ones, is far from being specific to our nematodes. Many such cases are known for a long time in other Metazoa. Wheeler, in a beautiful work on *Myzostoma*¹, gave us a rather complete list thereof, with the reference to the original work, in which each fact was described for the first time. Having few facts to add to the list of the American

¹ *Mittheilungen aus der zoologischen Station zu Neapel*, t. XII 1896, p. 290.

scientist, we refer to it without reproducing it here. It is indeed amply sufficient to show us the great extension of the phenomenon in the animal kingdom. Such cases were indeed observed in sponges, hydrae, platyhelminths, annelids, nematodes, mollusca, echinoderms, crustaceans and fish.

Cases of protogynous hermaphroditism, that is with the maturation of the female genital elements before the male elements, are in contrast very rare. Wheeler only cites those of the *Microstoma lineare*, of three slugs and of salps.

Protandrous dichogamy is also known to be so frequent in the vegetal kingdom that it is so to say the rule in most hermaphroditic plants. It is enough to recall here the fine works of Hildebrand, of Darwin, of Sprengel, that were devoted to the anatomical and physiological study of this disposition. We do not need to dwell on it any longer.

IV. - A fact, curious and common to all of our hermaphroditic nematodes, is the imperfection or rather the insufficiency of their hermaphroditism. One only needs to come back to the specific description of each of them (pages 23, 39, 51, 60, 70, 74, 80, 92, 98) and it will be seen that after laying for a few days fertilized eggs that developed regularly, all then continued for a duration two to three times longer to produce unfertilized eggs that fell rapidly apart. The average number of fertilized eggs varies between 200 to 250. In only one species (*Rhabditis Guignardi*), it rises to 520 to 530.

It is very obvious that the first stock of fertilized eggs corresponds to the number of spermatozoa produced by the genital gland, during its period of protandrous activity. As long as there are spermatozoa in the seminal receptacle of the uterus, the eggs that fall in its organ

are fertilized and complete their organization. The number of fertilized eggs laid per female informs us with certainty of the number of spermatozoa produced by the genital gland. Nevertheless, this number is very insufficient, since, after having exhausted their sperm, all our females continued to lay unfertilized eggs, in numbers two to three times greater than that of fertilized eggs. It must be concluded that there is a defect in harmony between the male and female activities of these hermaphrodites and that at least two thirds of their egg production are perfectly useless, since they are fatally destined to abortion and destruction.

Concerning reproduction, this incomplete hermaphroditism is far from being an advantage to the affected species. These nematodes, indeed, would have given birth in the dioic state to a progeny at least three times more numerous, composed of 700 to 800 individuals. Among these 800 individuals, half would have belonged to the female sex; thus, from the first generation on, the number of reproducing individuals would have been doubled. At the second generation, the number of dioic females would have reached 160,000, whereas that of hermaphrodites only rises to 60,000. It can therefore be seen what an enormous difference is found in the power of reproduction of these two modes of generation. There are certainly in the dioic state unfavorable accidents that prevent mating and fertilization of the females. But because nematodes always live in large and compact groups, these unfavorable accidents must occur infrequently. What is certain is that when one explores a rich culture of these rhabditids in full prosperity, all females are always found to be fertile.

We thus allow ourselves to conclude that the hermaphroditism of nematodes is not the result of an adaptation that would have been

grasped and fixed by natural selection. This state, far from being advantageous, rather appears harmful to the species. The principle of usefulness can therefore not have operated in its fixation and conservation. The causes that presided over its origin and its development must be searched elsewhere.

V. – In this summary, we talked until now about these females as if they were completely alone and as if no male would ever be encountered among them. The matter thus presented is not exact. The male sex is never completely absent and, when one takes the trouble to look for it, one can always be sure to find it represented by some individuals. We demonstrated it for each of the species that we studied, and here are, summarized in a table, these demonstrations with the proportion of males for 1,000 females, and the reference to the page where the details about each species can be found.

| | | | |
|-----------------------------|--------|-------------|-------|
| <i>Diplogaster robustus</i> | 0.13 ♂ | for 1,000 ♀ | p. 94 |
| <i>Rhabditis Guignardi</i> | 0.15 | — | p. 70 |
| — <i>dolichura</i> | 0. 7 | — | p. 80 |
| — <i>Caussaneli</i> | 1. 4 | — | p. 41 |
| — <i>elegans</i> | 1. 5 | — | p. 26 |
| — <i>coronata</i> | 5 | — | p. 86 |
| — <i>Perrieri</i> | 7 | — | p. 65 |
| — <i>Marionis</i> | 7. 6 | — | p. 53 |
| — <i>Duthiersi</i> | 20 | — | p. 60 |
| — <i>Viguieri</i> | 45 | — | p. 75 |

It can be seen from this list that the proportion of males can vary within rather large boundaries, depending on the species. In

Diplogaster robustus, only one for ten thousand females can be counted, whereas in *Rhabditis Vigueri*, one would find 450 for the same number of females. These two species form the extremes of the series; between them, the other species are positioned at several intermediate degrees. If we knew of a larger number of hermaphroditic species studied in the same manner, it is highly probable that these intermediates would proliferate and that we would find forms with even more males than in *R. Vigueri*, approaching the proportion of normal dioic species.

These males, which are so rare in some species and always quite uncommon, even in the species where they are found in the highest proportions, do not offer anything peculiar or abnormal. Nothing can be noticed in their structure or general organization that could make them be considered as malformed or badly constituted. From their size, from the proportions of their body and from all the details of their organization, they correspond on every point to the ordinary male type of dioic rhabditids. Their testis itself is constituted in a completely normal manner and, as we repeated it concerning each species, its products, the spermatozoa, are from their shape, their volume and their structure completely identical to that produced by the genital gland of the females during its period of protandrous activity. These very rare males are thus indisputably the perfect and normal representatives of the male type of our hermaphrodites. Indeed, as in the dioic forms, they can be distinguished in each species by constant differences in the conformation and dimensions of the bursa, as well as the number and layout of the papillae. They thus constitute for each of these forms the specific criterion, as required for these nematodes by some authors.

VI. – But whereas these animals, when examined in their structure and their morphology, represent genuine and complete males, it is not so when their sexual faculties and activity are studied. We already said and repeated, concerning seven species on which we could experiment: these males have almost completely lost all sexual instinct and appetite.

Here are indeed the results of these experiments as a table, in which are indicated the number of females and males that were brought in contact, the number of days that their common life lasted, the number of fertilizations and finally the reference concerning the details of each experiment:

| | ♀ | ♂ | Fertilizations | Days | Pages |
|-----------------------------------|-----|-----|----------------|---------|-------|
| <i>Rhabditis elegans</i> | 139 | 112 | 6 | 4 to 9 | 29 |
| — <i>Caussaneli</i> | 42 | 35 | 0 | 5 to 10 | 45 |
| — <i>Marionis</i> | 28 | 42 | 13 | 5 to 7 | 55 |
| — <i>Duthiersi</i> | 62 | 41 | 1 | 4 to 6 | 60 |
| — <i>Perrieri</i> | 26 | 35 | 0 | 5 to 7 | 65 |
| — <i>dolichura</i> | 12 | 5 | 0 | 6 to 7 | 81 |
| <i>Diplogaster robustus</i> | 4 | 2 | 0 | 4 to 5 | 94 |
| TOTALS | 313 | 272 | 20 | | |

We see from these figures that 313 females were put in contact with 272 males, for a duration of 4 to 10 days, and that only 20 females were fertilized by these 272 males. Moreover, regarding these 20 fertilizations, 13 belong to a single species, *Rhabditis Marioni*, whose hermaphroditism is notoriously incomplete, which explains the greater sexual activity of its males.

In the six other species, the sexual inertia and indifference of males have become almost complete. As we observed in each of these species, one hardly ever sees their males make any attempt at

mating. Having retained their high motor activity, they constantly move and circulate in the midst of females, colliding with them at every moment, but not caring about them more than if they were any inert bodies.

This indifference may seem surprising to those that know the sexual drive of male *Rhabditis* in normal dioic species. If, in a rather numerous culture, the two sexes of these species, first reared apart, are placed together, the males will immediately be seen searching for females, embracing them in the fold of their caudal extremity by coiling up around them, and a number of matings will be seen after a short lapse of time. These matings are actually easy to observe, because they last always rather long.

In our males of hermaphrodites, the sexual instinct, the reproductive sense, appears in contrast to be completely abolished. But since, on the other hand, we ascertained the complete and perfect state of their organism and morphology, we must conclude that we are in the presence of a psychic decadence that is not concomitant with a morphological regression.

This psychic decadence can be explained, so do we think, by the general hermaphroditic state of these species. This state must indeed tend to obliterate the male copulatory instinct, which is not any more transmitted hereditarily, since the few males that reappear so to say accidentally, do not partake any more to the procreation of the next generations. The paternal heredity is thus fully suppressed, to the benefit of the mere maternal heredity. When, following the return of an atavism, an individual that is morphologically built like a male reappears in a generation, this individual has the male sexual organization; yet it does not have male aptitudes anymore, and its nervous centers must function with the instincts and faculties of a

female. Regarding the sexual status, there is a disjunction between the morphological constitution and the psychic functions. In these nematodes, the female plays only a passive role during mating. She waits and receives the male in the most complete inertia, so that all active role belongs to the latter. The very rare males of our hermaphrodites, having lost all sexual stimulus, behave themselves with the inertia of true females.

VII. – What is the meaning of these males, which reappear unexpectedly and so rarely, without so to say any *raison d'être*, since they do not play any role for the reproduction and conservation of the species? We can only see in them atavistic expressions of their former dioic state. According to us, the primitive state of our hermaphroditic nematodes was dioic. Their hermaphroditism must have appeared subsequently and secondarily. Under the influence of unknown actions, the genital organ of females started to produce male and female fertilizing elements, which were self-sufficient. These reproductive elements, being all of female origin, must necessarily have had an ever increasing hereditary tendency to only reproducing the female type. From there, gradual suppression of the male form, and almost absolute atrophy of its faculties in its rare reappearances.

These males thus only exist as witnesses of a past state, and the qualification as *atavistic males* fits them perfectly. They survive in the biology of our hermaphrodites, as in some beings are seen to persist imperfect organs, now useless, which the absence of use has led to a more or less advanced state of regression. The reappearance of males is due to these phenomena of atavistic comeback that are so well known in the living world. Maybe it would be appropriate to

compare them to the males of Cirripeds, whose qualification of *complementary*, with which they are designated, is probably badly justified. Delage¹ actually already hinted that these so-called complementary males would rather merit the qualification of primordial, like those of the Sacculina. Indeed, all these males are more or less degenerate survivors of a primitive state, and we therefore qualified them of atavistic.

VIII. – This gradual elimination of the male type in our nematodes is further demonstrated in the cases of partial and incomplete hermaphroditism described for *Rhabditis Marioni* (pages 52 and 54), *R. Duthiersi* (page 60), and *R. Vigueri* (page 75). This incomplete hermaphroditism expresses itself through the existence of females, among which some are not hermaphrodites at all any more and some are only halfway, one of the ovaries producing both genital elements, whereas the second one produces only female elements. The number and proportion of such individuals that escaped hermaphroditism differ in the three species. In *R. Duthiersi*, I only encountered half-hermaphroditic individuals and in small number; in *R. Marioni*, individuals of both categories appeared more frequently, but still with a certain rarity of the completely unisexual females; finally, in *R. Vigueri*, one could find one or two completely unisexual individuals per 10 females.

These species are also those for which I observed the highest proportions of males: 8, 20 and 45 per 1,000 females. These males appeared to have better conserved their sexual faculties and their reproductive sense than those of other species. Indeed, whereas they are still quite inert in *R. Duthiersi*, they appear more ardent in *R.*

¹ *Archives de Zoologie expérimentale*, t. II, 1884, p. 704.

Marioni, and finally in *R. Vigueri*, all appear to have conserved a completely intact sexual instinct. In the latter species is thus found a mix of hermaphroditism and dioicy, where all individuals conserved their full reproductive faculties. In spite of this, hermaphroditism is still very predominant since it affects at least four fifths of the females, and the males only appear in the proportion of 45 to 50 for 1000 females.

IX. – It is clear that these forms with partial and incomplete hermaphroditism show us in action, so to speak, the course of the evolution that substituted hermaphroditism to dioicy in our nematodes.

Species that were first absolutely dioic saw the appearance of hermaphroditic females. The latter, more or less numerous at the beginning, lived together with unisexual females and a rather large number of males with still intact sexual faculties. Then occurred a mix of hermaphroditism and diocyt, without weakening or degeneration of any sort for all individuals of this association, made of three types of active members: hermaphroditic females, unisexual females and males. Later, hermaphroditism gaining ever more ground, the completely and partially unisexual females disappeared; males became extremely rare and the few reappearances of this sex even lost all aptitude for mating and fertilization. This association became thus reduced to a single active member, the bisexual hermaphroditic female.

It is henceforth obvious that, in our nematodes, hermaphroditism is of secondary origin and dioicy the original state. Delage, in his fine work on *Sacculina*¹, and Pelseneer² independently reached the

¹ Evolution de la Sacculine. *Archives de Zoologie*, t. II, 1884, p. 704.

² *Archives de Biologie*, t. XIV, 1895, p. 33-62.

same conclusion, the former for the hermaphroditism of crustaceans, the latter, for that of molluscs. Fritz Müller¹ and Montgomery², guided by theoretical considerations, developed the same idea.

This conclusion, however, is contrary to the common idea of the primordial origin of hermaphroditism. The bisexual state, considered as inferior and more primitive, would have been in this opinion that of all original beings, and the dioic system would have been established only in a later evolution, by atrophy of the male genital organ in the females and conversely of the female genital organ in the males. This viewpoint, adopted as a general and well grounded notion by many biologists, such as Huxley, Darwin, Haeckel and Claus³, found its most complete and precise formulation by Gegenbaur⁴.

This theory relies in our belief on three types of facts: 1° the frequency of normal hermaphroditism across the animal kingdom and its near universality in the vegetal kingdom; 2° the accidental and teratological reappearance of individuals with a more or less complete hermaphroditism in regularly dioic species; 3° the bisexual state observed in the genital primordium of some species during embryonic development. To these three sorts of facts can be added the notion, borrowed from phylogenetic ideas, according to which the simple must derive from the complex.

¹ Die Zwitterbildung im Thierreiche, in: *Kosmos*, t. II, 1885, p. 321-734.

² On successive protandric and proterogynic hermaphroditism in animals. *American naturalist*, t. XXIX, 1895, p. 528-536.

³ The latter, however, admits the secondary origin in Cirripeds (*Untersuchungen zur Erforschung der genealogischen Grundlage des Crustaceen-System*, Vienna, 1876, p. 90). The sexual system of Cirripeds, with its unisexual or hermaphroditic females and its various forms of complementary males, is even more complex than that of nematodes. Unfortunately, it is as yet only known morphologically, the functional value of its diverse categories of males not having been verified experimentally.

⁴ *Manuel d'anatomie comparée*, trad. fr., 1874, p. 64.

These facts, in our opinion, do not possess at all the phylogenetic meaning that is usually attributed to them. They do not prove that all these hermaphroditic forms, either normally, accidentally or in the embryonic stage, descend from functionally bisexual ancestors. For us, the hermaphroditic state does not correspond and has never corresponded to a phylogenetic phase in animal evolution.

These diverse cases of embryonic, accidental or normal hermaphroditism, are simply a new testimony in favor of the identity and equivalence of genital elements. They are indeed the logical and necessary consequence of this equivalence, to which we shall come back later. Under its primordial form, amphigonia, or generation by mating of two gametes, was performed between absolutely equal and alike partners. It is still found today under this simple form in some lower plants with isogamous conjugation. Later, with the progressive evolution of beings that resulted in a great complexity of structure, sexual dimorphism appeared in order to answer the new needs and necessities that were created by this complexity. This dimorphism was first apparent in the genital elements themselves, which differentiated into macrogamete and microgamete, ovum and spermatozoon. Despite the differences in conformation and volume, both kinds of products nevertheless remained equivalent to each other from the genital viewpoint. It results that they could be formed on a single individual or on distinct individuals, according to the particular needs and adaptations of the species. But regarding the general biology, there are not two opposed and distinct entities that would correspond to either a feminine or a male nature. The products and conformations that we separate by placing them in either of these two categories, are only secondary

manifestations of a unique primordial element, thus diversified in two directions, only to answer accessory needs.

This primordial element, the genital germ cell, thus retains its fundamental identity everywhere and always. It can at any time and in every circumstance be modified in any of the two directions that characterize it (male and female), so as to answer the special needs and adaptations of the species. By modifying itself, it carries along by correlation of development the whole suite of secondary sexual characters.

These two forms, these two modes of manifestation always exist in the latent state in each genital element during its formation. At this moment, each of them potentially possesses both sexual tendencies, or to be more exact, each of them is neutral and waits for the determining circumstance that will lead it in one or the other direction. This circumstance is set by adaptation, and the particular sexual constitution of the species. In some groups, this constitution is fixed in an irrevocable fashion and we then have the forms with an absolute and invariable sexual dioicy. In other groups, in contrast, this constitution remained more vague and so to say more malleable; we see then dioicy, hermaphroditism and even parthenogenesis occurring side by side, in neighboring species and even in representatives of the same species.

This juxtaposition of so diverse sexual states is for us the best proof that they do not result in their origins of any hereditary descent, that is that they do not correspond to any phylogenetic stage. How can we admit the intervention of heredity and phylogeny in the sexual system of a genus such as that of *Rhabditis*, where we know of species that are either fully dioic, or dioico-hermaphroditic, or with half hermaphroditic and half unisexual animals, and finally

even with accidentally hermaphroditic males? How can we admit it in the cirriped genus of *Scapellum*, in which are simultaneously found forms that are fully and absolutely hermaphrodites; others, hermaphrodites with small complementary males that are degenerated to various degrees depending on the species; others finally, with normal separate sexes? These so diverse and so complex sexual modes and states are and can only be isolated manifestations, independent of the dual potential that resides within each organism with an amphigonic generation. Each case developed on its own and does not correspond to an ancestral heredity that would link its origin to a primitive form, common origin of all similar cases.

From this viewpoint, it is easy to explain the sporadic appearance of normal hermaphroditism, with its many variations, in the whole animal kingdom. Similarly, accidental hermaphroditism, rudimentary hermaphroditism (Bidder organ in toads), embryonic hermaphroditism (bisexual embryos in the vertebrates of Laulanié¹ and in the insects of Heymons²), finally teratological hermaphroditism, can be understood as easily. These facts are only individual manifestations of the dual fundamental sexual potential.

It should then no more be discussed whether a case of hermaphroditism is *primary* or *secondary*, in the phylogenetic meaning of the words. This generation mode is inherent to all beings with an amphigonous reproduction. Being so to say hidden within the depths of the organism, sometimes it rests in the latent state, some other times in contrast it develops and blossoms under the influence of yet ill-known causes.

Let us summarize these considerations in the following conclusions. Hermaphroditism derives from the neutral primordial

¹ *Comptes rendus de l'Académie des Sciences*, t. CI, 1885, p. 393.

² *Zoologischer Anzeiger*, t. XIII, 1890, p. 451.

sexuality of genital organs. It can in all zoological groups unexpectedly arise without ancestral antecedents and develop more or less completely. Some of these groups, probably endowed with a less solidly fixed and more malleable genital constitution, seem to offer a more propitious ground to its blossoming. Nematodes and cirripeds are excellent examples thereof.

Some authors (Claus, Korschelt, Pelseneer) thought that there was a correlation between sedentary life and hermaphroditism. According to them, the bisexual state mostly appears under the influence of sedentarity. If all hermaphrodites did fertilize themselves, this hypothesis would so to say be self-evident. But self-fertilization does not seem to be the most usual case in bisexual animals. One therefore cannot see which necessary relationship could exist between these two conditions, since mating requires, in the end, the coming together of distinct individuals. In any case, what we know of hermaphroditism in nematodes is not in favor of this opinion. Even though self-fertilization is the absolute rule in the bisexual species, they however remained as agile and as motile as their dioic congeners.

X. — It is a very obvious fact in our nematodes that hermaphroditism has only developed in the feminine form of the species. This conclusion appears already clearly from the study of the genital organs of bisexual species. Indeed, in all, these organs take the structure and disposition that are typical of females and not of males. Moreover, mixtures of dioicy and partial or complete hermaphroditisms, as those described in *Rhabditis Marionis* and *R. Viguiieri*, are completely impossible to conceive with a hermaphroditism developed onto the male type.

This result is in complete agreement with those reached by Claus¹ on crustaceans, Delage² on *Sacculina*, Brock³ in fish and Pelsencer¹ in molluscs. All these observers, studying hermaphroditism in these diverse zoological groups, recognized that it was always grafted onto the feminine form of these animals. It thus appears that there lies a general law, that the bisexual state of the genital gland only finds a favorable ground for its development in the individuals that have already undergone a somatic sexual differentiation in the female direction.

Would this not be a hint that this sex has conserved in its nature and its genital constitution something more archaic, more primordial? Perhaps it represents better than the male sex the primitive form of the species, the neutral form before all sexual differentiation? Sexual dimorphism did not always exist with the degree of differentiation that is known to us today. It is highly probable that the females have better conserved the primitive form that was common to both sexes. The male differentiations would thus be of secondary origin compared to the female type. The former would derive from the latter. This viewpoint is actually in full agreement with the evolution of the genital elements, isogamy having necessarily preceded anisogamy. Moreover, an observation by Giard further argues in favor of this idea. When, in crustaceans, parasitic castration strikes males, they lose all male characters when molting and dress themselves in the female livery.

Male hermaphroditism is not in itself impossible. We indeed observed and described (p. 32) very odd cases in our *Rhabditis elegans*. Several males of this species, perfectly constituted and

¹ *Untersuchungen zur Erforschung der genealogischen Grundlage des Crustaceen-System*, p. 90.

² *Archives de zoologie expérimentale*, p. 704.

³ *Zeitschrift für wiss. Zoologie*, t. XLIV, p. 374.

organized as males, displayed to us testes with a clearly protandrous hermaphroditism, like the genital organ of their sisters. The ova that were produced by these testes had in all details of their structure and organization a perfectly normal appearance, identical to that of the ova produced by females.

Would these ova of male origin have been capable of fertilization and development? Unfortunately I could not assure myself thereof. But I am ready to accept this possibility. They appeared as well constituted as the ova of female origin. Then one cannot see why they would not have enjoyed the same developmental properties as the latter, born like them in a genital gland that also first operated as a testis. The morphological identity and the identity of origin appear to me to necessarily result in an identity of faculty.

It is actually not the first description of the production of ova by testes with a complete male differentiation. Indeed, Hermann ², while studying sections made on the anterior end of a testis from a lobster, found eight to ten large ovoid cells, endowed with a large vesicular nucleus. He interprets these cells as ova; it results that the testis of this lobster is partially hermaphroditic. Similar facts were described in *Orchestia* by Nebeski, in *Gebia* by Ishikawa, in crayfish by Lavalette-Saint-Georges, in *Asterias glacialis* by Cuénot, in cladocerans by Kurz, in batracians by Friedmann, etc., etc. ³.

XI. – These females of protandrous hermaphroditic nematodes, the ovary of which successively produces spermatozoa and ova, these males of nematodes, of crustaceans and of batracians in the testes of which well-constituted ova can be found, all these facts and others

¹ *Archives de Biologie*, t. XIV, 1895, p. 58.

² *Bulletin scientifique de la France et de la Belgique*, t. XXII, 1890, p. 43, pl. III, fig. 7.

³ See: *L'Année biologique* 1896 et CUÉNOT in *Bulletin scientifique de la France et de la Belgique*, t. XXXII, 1899, p. 522, 523.

that we exposed elsewhere¹, provide the proof for the theory that states the identity of the male and female sexual elements. This theory, proposed by Leuckart², accepted by Balfour, by Spencer³, is today quite indisputable. The so large differences in shape and volume between these elements are only accessory differentiations, which only affect the secondary parts of their organism and are destined to facilitating their encounter and copulation. But the essential part, the germinal nucleus, is identical in both elements. The male nucleus and the female nucleus have an absolute morphological and physiological equivalence. This was well demonstrated by the facts just cited, where we see them originating from a same unique genital gland.

XII. – We summarized above the experiments in which we tried to have hermaphrodites having exhausted their own sperm be refertilized by their rare males. We saw that these attempts succeeded in only three species, *Rhabditis elegans* (p. 29 and following), *R. Marionis* (p. 54) and *R. Duthiersi* (p. 60). When describing each of these species, we gave the detail of these refertilizations, which we call heterogamous, by opposition to the autogamous fertilizations that result from the sperm produced by the hermaphrodites themselves.

The eggs thus obtained by heterogamous fertilization gave birth in the two latter species to individuals that did not differ in anything from the individuals with an autogamous origin. The heterogamous generations were indeed composed of hermaphroditic females, accompanied by some rare males in the usual form and proportion.

¹ *Archives de zoologie exp. et gén.*, t. VII, 1889, p. 455.

² Article: *Zeugung*, in *Wagners Handbuch der Physiologie*, p. 807.

³ *Principles of biology*, 2nd edition, t. I. 1898, p. 282.

But it was not so with *Rhabditis elegans*. We indeed saw when describing this species (p. 29 and foll.) that the heterogamous fertilization had a strong arrhenotokous influence on its products. The proportion of males that, in the autogamous generations, was of 1 to 2 per 1,000 females, rose to 463 per 1,000, that is to the usual figure in the dioic *Rhabditis*, thus restoring the equilibrium between both sexes. But we also saw that these males of heterogamous origin seemed even less endowed of reproductive sense than their autogamous brothers and that, whereas heterogamy exerts a very clear and obvious action on the formation and production of male individuals, it certainly does not exert any on the conservation and expansion of the species. These males, well constituted and organized in their shapes and morphological structures, are completely devoid of any sexual instinct. They never mate and thus do not play any role in the general life of the species.

In any case, this arrhenotokous influence of heterogamous fertilization is not less interesting. As we said, it is a kind of counterpart to the thelytokous fertilization of bees and brings to us a new case of sexual determinism within the egg proper and outside any kind of trophic influences. As we saw, Brauer already ascertained such an arrhenotokous action of fertilization by the males of the parthenogenetic females of *Apus*. In these three cases, bees, nematodes and *Apus*, the sexual determinism ensues only from fertilization and is consequently fully independent from the influences of age, environment or nutrition. Moreover, and curiously, in one case it is thelytokous, in the others arrhenotokous.

XIII. – As we could see in pages 27 and 42, I nevertheless wanted to verify with *Rhabditis elegans* and *R. Caussaneli* whether age or

modified poorer nutrition would not have an influence on the proportion of the sexes in these two species. The results were absolutely negative. Old or young, well or ill-fed, our hermaphrodites continued to lay females and males in the ordinary proportion. The only difference was in the total number of laid eggs, higher in the well-fed than in the ill-fed animals¹.

These experiments and all the facts that we described concerning the different sexual states of our hermaphrodites throw some light on the determinism of sexuality in these animals. But, in order to understand them well, it is good to remember that we are here in the presence of a double determinism, that of the generative individuals (males and unisexual or hermaphroditic females) and that of the reproductive elements (oocytes and spermatocytes). It is indeed certain that the cause that determines the production of a hermaphroditic female or of one of its very rare males does not operate in the same conditions than that which will condition the appearance, in the genital gland of these females, first of spermatozoa, then of ova. The first acts upon entire organisms, to which it imparts a particular character and sexual structures, leading, for some of them in the masculine direction, for the others in the feminine direction. The latter has a much more restricted action, limited to the mere genital elements.

This cause, whether single or double, must operate in our hermaphrodites successively and in conditions that are independent of each other, first setting the sexual morphology of the individuals, then acting on their genital gland to make it produce, one after the other, both types of generative elements. This succession and this independence of action manifest themselves clearly in the partial

¹ Cuénot arrived at identical results when experimenting with *Calliphoria vomitoria*, *Bulletin scientifique de la France et de la Belgique*, vol. XXXII, 1899, p. 480.

specific hermaphroditism, as we described it in *Rhabditis Vigueri*, and above all in the incomplete individual hermaphroditism observed in some females of *Rhabditis Duthiersi* and *R. Marionis* that are half hermaphrodites and half unisexual. The males of *R. elegans* with a hermaphroditic testis lead to the same conclusion.

It is indeed indisputable that the cause that fixes the proportion of sexes and gives rise to a large majority of females in a generation of *Rhabditis Vigueri* is not the same, or at least does not act under the same conditions than that which, given these females, transforms some into hermaphrodites and others into unisexuals. We certainly find ourselves facing different actions and influences when we see one half of the genital organ being hermaphroditic, the other half unisexual, as in some females of *R. Duthiersi* and *R. Marionis*. The cause leading to this dissogony of the genital apparatus cannot be acting under the same conditions than that which determines the general morphology of the animal. This distinction is also obvious concerning the hermaphroditic males of *R. elegans*. We must thus admit in our hermaphroditic nematodes, let us repeat it, the existence of a double sexual determinism, each acting successively and independently of each other.

This being well established, what are the determining actions and at which time do they exert themselves?

Regarding their timing, all what we have learnt of the biology of these beings leads us to ascertain that it is in the ovary, before any further development, that the future sex of its products is fixed. If it were not the case, we could not understand how, out of the hundreds and thousands of these eggs that are laid by these dioico-hermaphroditic nematodes, an enormous proportion of females, so constant and so overwhelming, should come out, when, in contrast,

in the species with distinct sexes, the sexual equilibrium is maintained as regularly. When we tried to see whether age or nutrition could modify the sexual system of these animals, we observed that these factors did not have any influence. We must thus admit that the sex is predetermined in the very young reproductive element and that no exterior influence could have any modifying action onto it anymore.

In a single case could we act on the nature of the sex of the products. This concerns the heterogamous refertilization of old females of *Rhabditis elegans*; refertilization with a modifying arrhenotokous influence that was in fact only partial, since it simply led to a restoration of the equilibrium between both sexes. But this very case also brings us back to the precocity of fixation of the sexes, since it is at the time of fertilization that the modifying action of the spermatozoa of heterogamous origin makes itself felt. These spermatozoa, by uniting with ova with a predetermined feminine tendency caused immediately and irreversibly the appearance of the opposite tendency in half of them.

The dissogonic females of *Rhabditis Duthiersi* and *R. marionis*, with one hermaphroditic genital gland and the other unisexual, lead us again to the same conclusion. The hermaphroditism of the former and the unisexuality of the latter must date from the very origin of these apparati, when they are still represented by a common primordium, formed by two large germinal nuclei that are identical in appearance (pl. XVII, fig. 10). We cannot see indeed how these two glands, separated by hardly a millimeter and enclosed within the common general cavity, could have been subjected during their development, independently of each other, to external influences that would have made them follow so different sexual developments.

This viewpoint is further corroborated by the fact that, in these dissogonic females, the hermaphroditic gland always appears to produce the quantity of sperm that is proper to the species. They thus in reality have nothing abnormal nor monstrous.

As a last argument in favor of the precocity of sexual determinism, let us also cite the regularity and the suddenness with which the genital apparatus goes from the masculine to the feminine tendency. The hermaphroditic glands indeed produce a quantity of sperm that is always approximately the same and depends on each species; then, all of a sudden, without a halt and without transition, they start making ova. This obviously applies to normal, well-constituted and well-fed animals. It is not the same for weak individuals. The number of genital elements that they produce can be strongly reduced and I believe that with a bad nutrition, given with the proper timing, one could completely suppress the production of spermatozoa, whereas that of ova would only be more or less strongly diminished. In the normal individuals, this regularity and this suddenness in the change from spermatogenesis to oogenesis cannot depend upon external causes. Indeed, such causes necessarily act with variable intensities on different individuals. They could not produce as regular and clear-cut effects as the ones we know of. We must thus admit that, from their first origin and their first rudimentary primordium, the genital apparatus of our hermaphrodites bear with them in a latent state all the conditions of their later functioning.

Whichever direction we turn to, and questioning all the facts at our disposal, we always end up with the same conclusion: the sexuality of the individuals on one hand, that of the reproducing elements on the other hand, are irrevocably predetermined from the

time of maturation of the first embryonic germinative cells whence they derived. From then on, no influence of age, environment or nutrition can act upon them and modify and transform their sexual characteristics.

Almost ten years ago, I had reached an identical result, when studying the same phenomena in *Hydatina senta*¹. Since, these ideas made their way, and Cuénot, in an excellent work that was published very recently², has successfully tried to generalise and extend them to the whole animal kingdom. Reexamining prior works that pretended to demonstrate a later sexual determinism, and submitting them to a clever and penetrating criticism, even performing again some of the conflicting experiments that had until then appeared most decisive, the learned professor from Nancy could conclude that “the sex is irreversibly determined in the egg, and at the latest when this egg is fertilized. In no case could one demonstrate, in a decisive manner, the presence of a factor acting after fertilization”. The facts observed in our nematodes fully match these general ideas.

It remains now to examine the other part of the question that was raised above: What are the actions that determine the sex?

Here we face things that are much more obscure and difficult to grasp. As we saw, the few experiments that were performed to modify the sexuality of our hermaphroditic nematodes all yielded negative results, except for one. The differences in age, environment and nutrition had no effect on the sexual nature of these nematodes. The only case of the old females of *Rhabditis elegans* that were

¹ See MAUPAS, *Sur le déterminisme de la sexualité chez l'Hydatina senta*, in Comptes rendus de l'Académie des Sciences, t. CXIII, 1891, p. 338. – Some criticisms on facts and interpretation have been aimed at this provisional work. I have the hope to be able one day to reexamine it in complete detail and demonstrate the lack of ground for most criticisms.

² CUÉNOT. *Sur la détermination du sexe chez les animaux*, in *Bulletin scientifique de la France et de la Belgique*, t. XXXII, 1899, pp. 462-535.

refertilized with sperm of heterogamous origin and became partially arrhenotokous, as interesting as it can be, is very particular and without a general scope. The similar refertilizations that were obtained in *Rhabditis Duthiersi* and *R. Marionis* did not indeed produce the same result. The sexual system of these nematodes appears so solidly and so deeply rooted in their organic constitution, that it so far escapes all our means of action. In any case, we are still in the most complete ignorance of its determinism.

It is actually almost the same for all other animals, save for the few rare cases where the operation of fertilization (*bees*, *Apus*, *Rhabditis elegans*), of heat (*Hydatina senta*) and nutrition (*aphids*) appears well established. Everything leads me to believe that the importance of the latter of these factors has been strongly exaggerated, being called for in a number of cases that had nothing to do with it. For many authors, the determinism of sexuality would be a mere question of nutrition; plentiful food favoring the production of females, and, conversely, a lack of food favoring that of males. Cuénot has put together many objections to this theory and nothing would be easier than to add new ones. But we have no intention to enter here into a thorough discussion, and we will satisfy ourselves with examining it from the viewpoint of our hermaphroditic nematodes.

The formation of their male genital elements occurs when these animals have just reached the adult state; that is, the period of their greatest physiological activity. These animals are then in full possession of their faculties in their entire freshness and plenitude. In no other period of their existence will the functions of nutrition play with greater perfection and efficiency. Then comes the period of ovulation that begins when the organism is still in its full vigor. This

period extends and continues until the end of life, that is, finally, during a period where all functions by gradually weakening and slowing down end up leaving the animal with only a lessened and withering life. If the theory of the influence of nutrition was correct, it is at this time that the period of spermatogenesis should take place, and not, as we saw on the contrary, at the time of its brightest activity. Similarly, the testis of the hermaphroditic males of *Rhabditis elegans* produced sperm during the vigorous youth of these animals and then started to form ova when they began to decline towards old age.

Let us thus repeat it, everything, or almost everything, is yet to be discovered concerning the causes and conditions that preside to the production of the sexes. The only well-ascertained point at this day is that these causes are diverse and that each case will have to be studied for itself, without us being able to conclude from one species to the other. Indeed, nothing better demonstrates this diversity than when we see fertilization in one case determine thelytoky (*bees*), in other cases arrhenotoky (*Apus, Rhabditis elegans*).

XIV. – Another, as interesting, question was whether our hermaphroditic nematodes could maintain and reproduce themselves indefinitely by mere self-fertilization and whether their generations would always remain vigorous and fertile in this full inbreeding. On pages 33 and 45 can be found details about the cultures undertaken with *Rhabditis elegans* and *R. caussaneli* in order to verify this biological problem.

Three cultures of autogamous generations were organized and maintained, one until the 41st generation, the second until the 49th, finally the third one until the 52nd generation. All became

extinguished following degenerations that translated into either abortion of the embryos, which failed to hatch, or into the withering of the larvae, which, puny and unable to feed, could not finish their development and reach the adult state; or finally into the full sterility of the genital organs of the few individuals that succeeded in completing their development. These degenerations appeared suddenly in my cultures, and always when the ambient temperature rose to 23° to 24° C. I concluded that the rise in temperature appeared to be the cause of extinction of these cultures and that autogamy was not at all involved. I however do not consider my experiments as absolutely definitive. They should be undertaken with arrangements that would allow to spare the animals temperatures that appeared to me to be the cause of their degeneration.

In any case, the result of these cultures that were maintained for many generations in the strictest self-fertilization, together with the well-established fact of the sexual incapacity of the males of our hermaphrodites, these facts are a great stumbling block for the theory that states the absolute and universal necessity of cross-fertilization. This theory, at a given time, had almost reached the state of a dogma, and Darwin, following Conrad Sprengel, had come to declaring: "Nature thus tells us, in the most emphatic manner, that she abhors perpetual self-fertilisation," and elsewhere: "No hermaphrodite fertilises itself for a perpetuity of generations¹."

That cross-fertilization has in many cases its advantages, is impossible to deny, after the admirable works of Darwin and his followers. But that it be always and everywhere fully necessary to the maintenance and conservation of species, is an erroneous

¹ DARWIN. *On the various contrivances by which orchids are fertilized*, p. 359, and *The Variation of Animals and Plants under domestication*, 2nd edition, p. 159.

exaggeration. Already the American botanist Meehan¹ formulated serious reservations against this doctrine, reservations that were grounded on observations that were contradictory to those of the illustrious English scientist. Moreover, Darwin himself, with the sincere honesty that characterizes his work, cites several experiments and observations opposing his conclusions and confesses the existence of plants for which self-fertilization not only does not cause any damage, but even appears to have advantageous effects².

We are convinced that with the progress of researches, these cases will grow in number. The self-fertilization of our protandrous hermaphroditic nematodes certainly does not constitute an extraordinary and isolated exception. In any case, its mere existence would suffice to counter the exaggerated statements cited above. But Wheeler had already recognized³ that in *Myzostoma glabrum* the directly fertilized eggs developed as well as those that were fertilized by sperm of foreign origin. Many other hermaphrodites are very probably in the same case. Protandry is not at all an adaptation developed to avoid self-fertilization; rather, as Wheeler says very well, it is the result of the physiological development of the genital elements, the male elements having, through still ill-known causes, almost always a more precocious development than the female ones.

We must therefore admit that the crossing of the fertilizing elements is useful and even necessary in some cases; but that in other cases, it is indifferent and can even be advantageously replaced by direct fertilization. These two modes of genesis are thus only

¹ MEEHAN. *On self-fertilization and Cross-fertilization in Flowers*. Penn. Monthly, t. VII, 1876, pp. 834 to 843. Cited after GEDDES and THOMSON, *L'évolution du sexe*, fr. transl. 1892, p. 104.

² DARWIN. *Des effets de la fécondation croisée et de la fécondation directe*, fr. transl. 1877, pp. 353-357, 450 and 471.

³ *Mittheilungen ans der zoologischen Station zu Neapel*, t. XII, 1896, p. 291.

peculiar adaptations of oogenetic reproduction and, like all biological adaptations, are subject to variations. That the mode through crossing is the most widespread, that it even enjoys some particular characteristics, seems to stem from the present state of our knowledge. But it does not for all that exclude completely the more primitive and simple mode of self-fertilization. Both coexist in parallel and are necessary or indifferent depending on the cases.

XV. – Before turning to the parthenogenetic species, let us recall that in *Diplogaster minor* we observed and described (p. 99) eggs with a sort of a beginning of parthenogenetic development. These unfertilized eggs could develop until the morula stage, then stopped and disorganized themselves. It seemed to us that we had there a hint of the path followed by the evolution leading from hermaphroditism to parthenogenesis. It is very well possible that the parthenogenetic species derive from autogamous protandrous hermaphroditic species and we would not be surprised if one would come to discover a new type of these hermaphrodites that, after having exhausted their stock of sperm, would nevertheless continue to lay eggs developing regularly through parthenogenesis, instead of laying unfertilized eggs, as do those that we presently know. One would thus find, in this type of transition, hermaphroditism and parthenogenesis joined in succession, as we have already seen half bisexual and half unisexual hermaphrodites¹.

The seven parthenogenetic species that we could study belong to seven different genera. Parthenogenesis, less frequent than

¹ Analogous beginnings of parthenogenetic development were already mentioned several times for very diverse animals; in pig by Bischoff (1844), in the Firola by Vogt (1852), in frogs by Leuckart (1853), in rabbit by Hensen (1869), and in hen by Oellacher (1872). In silkworms, all degrees of parthenogenetic development have been observed, from the morula to the formation of adult individuals that hatched normally. All these facts prove to us that the animal egg can probably in many cases enter its development without the participation of the spermatozoon.

hermaphroditism from the species viewpoint, on the contrary appears to have a greater generic scope. Here is the list of these seven species, with the reference to the page where each of them was studied:

| | |
|-----------------------------------|--------|
| <i>Rhabditis Schneideri</i> | p. 99 |
| <i>Cephalobus dubius</i> | p. 102 |
| - <i>lentus</i> | p. 110 |
| <i>Plectus cirratus</i> | p. 115 |
| <i>Aphelenchus agricola</i> | p. 119 |
| <i>Alaimus Thamugadi</i> | p. 123 |
| <i>Macrolaimus crucis</i> | p. 126 |

Despite the most careful research, I have never seen the sign of males in these nematodes. But it must be added that most of them lend themselves with difficulty to mass cultures, either because they completely refuse to live in captivity, or because they multiply too slowly. It was thus impossible for us to obtain numerous masses thereof as for the hermaphroditic forms, and our study has inevitably been limited to restricted numbers of individuals. Only *Rhabditis Schneideri* could be greatly multiplied, and I could count 4,039 adult individuals, raised in special cultures, without encountering a single male. In the many long cultures of *Cephalobus dubius* that I made, many hundreds of individuals that were all females passed in front of my eyes. If males sometimes reappear in these species, they must be very rare.

I am actually well disposed to admit of their existence. Parthenogenesis in these nematodes is certainly of secondary origin, and the species that have adopted this mode of generation did derive from dioic species, either directly, or through the intermediate of hermaphroditic forms, as we explained above. Then, it would be very

natural that, through an atavistic phenomenon, males would reappear now and then in the generations of parthenogenetic nematodes, exactly as we have seen them reappear in the forms with even the most complete and absolute hermaphroditism. The extremely rare males of *Plectus cirratus*, which De Man illustrated and described, seem to be an empirical demonstration of this viewpoint. Therefore we believe in the difficult yet certain discovery of males in parthenogenetic species; males that most probably will show themselves as inert and useless as those of the hermaphrodites.

Regarding *Cephalobus dubius*, we mentioned the existence of clearly distinct and permanent local races. We demonstrated their fixity in extended cultures. We refer to the particular description (p. 109), where these facts are described in detail, with the important consequences deriving thereof.

XVI. – To end this overall review, it can be summarized in a few words. Oogenesis in nematodes has taken all the forms that this mode of generation is capable of taking. We find, indeed, perfect dioicy, hermaphroditism mixed with dioicy, then complete and absolute hermaphroditism, and finally, at the top, parthenogenesis. The course followed in this evolution is indicated by the constant presence of males, which, numerous and very active in the primary states and phases, become ever rarer and finish by totally loosing the sense of reproduction in the terminal states and phases. All these dispositions, which are apparently so different from each other, are however condensed and accumulated in very closely related forms and in very narrow specific frameworks. They form one more demonstration of the ease and flexibility with which the genital

elements can be modified and varied, without the vegetative part of the individuals being the least affected. In other words, the life of the individual and the life of the species appear to be completely independent of each other, and their respective organs vary and are modified each in its direction, without any reciprocal correlation or repercussion. Of course, we only allude here to specific forms and not in the least to the secondary sexual characters, which are directly under the dependence of the state of the genital organs.

If we take into account, on one hand this ease to vary that characterizes the genital elements, on the other hand the fact that hermaphroditic species belong to genera where dioic forms are as and even more numerous, one is led to admit that each hermaphroditic type has appeared and developed independently of the others and that no phylogenetic filiation exists between them. In other words, each hermaphrodite derives from a preexisting distinct dioic form. The partial and incomplete hermaphroditism of some species and the constant existence of a few rare males with a well-defined specific constitution also come in support of this viewpoint.

It thus results that these hermaphroditic species are perhaps only local races with a more or less spread geographic expansion, and that future research will probably discover somewhere the dioic form of some of them.

APPENDIX

ON MOLT AND ENCYSTMENT

When I gave my work on *Molt and Encystment in the Nematodes*¹ to print, I had not had the opportunity to consult the important report of Leuckart on *Allantonema mirabile* and *Sphaerularia*

¹ *Archives de Zoologie expérimentale*, 3^e série, tome VII, 1899, pages 563-628, plates XVI-XVIII.

*bombi*¹. Having since been able to obtain this work, I found therein excellent observations, which perfectly agree with mine and serve them as controls. Reciprocally, the facts described by the German scientist, examined and criticized in the light of my own observations, are placed without difficulty within the developing framework that I reached. I thus thought that it would not be pointless to devote here a few lines to this critical examination.

Leuckart mentions (p. 589) a first molt in *Allantonema mirabile*, molt that must probably occur shortly after hatching. Later, when the larvae have grown of two thirds of their original size, he finds them enclosed under a cuticular envelope (p. 591, fig. 9), which, from the description he gives us, completely matches a cystic envelope. These larvae, indeed, can live for a long time without change, enclosed within this cyst, and migrate into the general cavity of their host to lodge themselves under its elytra. There is no possible doubt, we have here true encysted larvae at the end of their second stage of development, following the law of development that we established. This cyst thus corresponds to the second molt in our framework of exuviations.

Leuckart then saw these larvae deencyst (p. 594), grow and reach the sexual adult age. These informations on the third and fourth molts are incomplete and quite unclear. He however mentions (p. 601) adult individuals wearing a double exuvial cast. They very probably correspond to the last two molts.

In *Sphaerularia bombi*, the learned professor did not observe the first molt. However, he describes to us with the most appropriate and perfect details the encysted state. According to him, the cyst (p. 630) is impermeable to coloring substances, as we have observed in

¹ *Neue Beiträge zur Kenntnis des Baues und der Lebengeschichte der Nematoden*, 1887.

Rhabditis elegans. The encysted larvae have a very developed migratory instinct and their aspect is thin and shrunken. They can live without a change for weeks and months enclosed in their cyst (pp. 630, 631, 634). They retract in their cyst and undergo their third molt in its cavity (p. 635). Finally their fourth and last molt occurs (p. 645) when their vagina and their uterus begin to form an outside hernia.

All these facts thus agree very well with the framework of the molts and developmental stages as we established them in our study. It is interesting to find again the confirmation of it in a work that is earlier by more than twelve years and devoted to animals so different by their biology as those that were used in our observations.

I believe that to the list that I gave ¹ of cases of encysted larvae known so far, the larvae of *Filaria sanguis hominis* should be added, as Lewis made them known to us ². These larvae, which live enveloped in a cuticular and persisting sheath without change and development in the blood of patients affected with filariasis, could only be encysted larvae. Under this form of latent life, they wait for their transfer in the appropriate and final environments, where they will continue and complete their development. According to the latest researches, mosquitoes and water would be the agents of transmigration, and the human lymphatic system their final milieu of choice.

I am also very inclined to believe that we are again in the presence of encysted larvae in all parasitic species with migrations (*Ollulanus*, *Spiroptera*, *Sclerostomum*, *Trichina*, etc.), the juveniles of which encapsulate in the tissues of the temporary host. All these

¹ *Archives de Zoologie expérimentale*, 3rd series, t. VII, 1899, p. 625.

² See LEUCKART, *Die menschlichen Parasiten*, 1876, p. 632.

encapsulated larvae enjoy properties of resistance and of suspended life that are characteristic of encysted larvae. I am thus convinced that, by properly studying these species, one would recognize that they are all at the end of their second stage of development. If my supposition was confirmed, the encapsulation would only be an accessory phenomenon, being linked to encystment as an ensuing epiphenomenon. Following the encystment of these nematodes inside their temporary hosts, encapsulation thus becomes so to say fatal, and the second process is merely the consequence of the first. Their distinction is thus very clearly established and does not allow their confusion under the same denomination.

Moreover, the development of these parasites appears under a new light. It is related without difficulty to that of the free-living nematodes, since it follows all the stages of it with only few accessory modifications caused by the difference between environments. Here is, it seems to us, an interesting viewpoint that would merit to be completely elucidated by the observers who devote their works to the study of parasitic nematodes.

These helminthologists will also find in the knowledge of our stages of development a guide to direct them in the study of the complete development of the species with migrations, whose existence is shared between different hosts and environments. They will have above all to try to grasp well the conditions and the environments in which these species undergo their second stage and finally encyst. For I am convinced that only in the encysted state can the migration occur with success and the infestation of hosts take place, in which these parasites complete their development. Thus, in order to well understand the mode of dissemination and infestation of the Strongylids that devastate our herds of ruminants through the

bronchitis and pneumonia that their presence in the adult state determine, one should before anything else know exactly the conditions of encystment of these parasites. These conditions once recognized, the prophylaxis of these wormy epizootic diseases would very probably become simple and of an easy execution. It is by protecting our domestic animals against the encysted larvae and destroying the first and second stage larvae in the environments in which they live that we will succeed in restraining the propagation of these illnesses.

(*Note at page 132, line 9 from the top.*) I can already confirm my predictions concerning the *Dorylaimus* genus. During the last days, I have encountered many adult examples of *Dorylaimus Bastiani*. All were females. Having isolated them, these females laid regularly developing eggs. I have then examined with the greatest care their genital organ and could not discover in it any sign of spermatozoa. At this day, about fifty species of *Dorylaimus* have been described, a good half of which are only known through females, males having never been encountered. It is highly probable that the great majority of these species without males are either parthenogenetic or hermaphroditic. We actually saw that Schneider considered his *Dorylaimus (Enoplus) liratus* as hermaphroditic.

EXPLANATION OF PLATES

PLATE XVI

- Fig. 1. Adult female of *Rhabditis elegans*: *v*, vulva; *c*, nerve ring and excretory pore; *a*, anus; *u*, uterus; *o*, oviduct(tuba); *g*, ovary. Mag. 150.
2. Adult male of the same. Mag. 150.
3. Section of the body with the cuticle separated in two under the action of acetic acid at 1 o/o. Mag. 335.
4. Mouth, buccal cavity and anterior extremity of the esophagus. Mag. 1510.
5. Esophagus: *c*, nerve ring; *p*, excretory pore. Mag. 335.
6. Female tail: *a*, anus; *p*, caudal papilla. Mag. 335.
7. Oviduct *o*, linking the vitellogen *v* to the uterus, *u*. The oviduct swollen like a bag serves as a seminal reservoir; *i*, intestine. Mag. 335.
8. Germinal cells lining the ovary sheath as an epithelium and enveloping an amorphous rachis. Mag. 800.
9. Blind end of the ovary: *cc*, terminal cells and cells of the intima; *g*, germinal cells. Mag. 800.
10. A, B. Bursa and spicules seen fullface and sideways: *i*, intestine; *c*, deferent canal; *g*, rectal gland. Mag. 395.
11. Spermatozoa. Mag. 1510.

PLATE XVII

- Fig. 1. Testis of *Rhabditis elegans*: *s*, spermatoblasts; *sp*, spermatozoa; *b*, bursa and spicules; *c*, deferent canal; *i*, intestine. Mag. 335.
2. Hermaphroditic testis of the same: *ov*, ova; *sp*, spermatozoa. Mag. 335.
3. Young *R. elegans* having just hatched: *g*, genital rudiment; *c*, nerve ring and excretory pore; *a*, anus. Mag. 705.
4. Adult female of *Rhabditis Caussaneli*: *v*, vulva; *u*, uterus; *o*, oviduct; *g*, ovary; *a*, anus. Mag. 58.
5. Mouth and buccal cavity. Mag. 1330.
6. Esophagus: *c*, nerve ring and excretory pore. Mag. 270.
7. Female tail seen fullface: *p*, caudal papillae. Mag. 270.
8. Eggs with already advanced embryos: *g*, polar body. Mag. 270.
9. Nuclei of the intestine. Mag. 1510.
10. Genital rudiment at the time of hatching. Mag. 1510.
11. Spermatozoa. Mag. 1460.

PLATE XVIII

MODES AND FORMS OF REPRODUCTION OF NEMATODES. 171

- Fig. 1. A, B. Male tail of *Rhabditis Caussaneli* seen frontface and sideways: *i*, intestine; *c*, deferent canal; *g*, rectal glands; *s*, spicules and gubernaculum (accessory piece). Mag. 395.
- Fig. 2. Juvenile of the same having just hatched: *r*, genital rudiment. Mag. 335.
3. Adult female of *Rhabditis Marionis*: *v*, vulva; *o*, oviduct; *g*, ovary; *a*, anus. Mag. 80.
4. Anterior end, mouth and buccal cavity. Mag. 810.
5. Esophagus: *c*, nerve ring: *e*, excretory pore with its ascending and descending canals; *g*, unicellular gland. Mag. 335.
6. Female tail: *a*, anus; *g*, rectal glands; *p*, lateral papilla. Mag. 335.
7. A, B. male tail seen fullface and sideways: *i*, intestine; *c*, deferent canal; *g*, rectal glands. Mag. 395.
8. Spicule and gubernaculum (accessory piece) seen sideways. Mag. 1280.
9. Eggs at the six-blastomere stage. Mag. 335.
10. Spermatozoa. Mag. 1460.
11. Juvenile having just hatched: *r*, genital rudiment. Mag. 335.
12. Spermatozoa of *Rhabditis Duthiersi*. Mag. 1460.
13. Nuclei of spermatozoa of the same treated with acetic acid. Mag. 1460.

PLATE XIX

- Fig. 1. Adult female of *Rhabditis Duthiersi*; *v*, vulva; *o*, oviduct; *g*, ovary; *a*, anus. Mag. 80.
2. Anterior end, mouth and buccal cavity. Mag. 395.
3. Esophagus: *c*, nerve ring; *p*, excretory pore. Mag. 335.
4. Female tail: *g*, rectal glands; *a*, anus; *p*, lateral papilla. Mag. 335.
5. Eggs at the two- and six-cell stages. Mag. 335.
6. A, B. Male tail seen fullface and sideways: *i*, intestine; *c*, deferent canal; *g*, rectal glands. Mag. 395.
7. Spicule and gubernaculum (accessory piece) seen sideways. Mag. 1280.
8. Adult female of *Rhabditis Perrieri*: *v*, vulva; *g*, ovary; *a*, anus. Mag. 85.
9. Anterior end, mouth and buccal cavity. Mag. 700.
10. Esophagus: *c*, nerve ring; *p*, excretory pore. Mag. 335.
11. A, B. Female tail seen fullface and sideways: *g*, rectal glands; *a*, anus; *p*, lateral papillae. Mag. 335.
12. Egg at the two-blastomere stage. Mag. 335.
13. Male tail seen fullface, with its bursa, its papillae and its spicules. Mag. 705.
14. Spermatozoa. Mag. 1510.

PLATE XX

- Fig. 1. Male tail of *Rhabditis Perrieri* seen sideways: *i*, intestine; *c*, deferent canal. Mag. 705.
2. Adult female of *Rhabditis Guignardi*: *v*, vulva; *a*, anus; *g*, ovary. Mag. 85
3. Anterior end, mouth and buccal cavity. Mag. 800.

4. Esophagus: *c*, nerve ring; *p*, excretory pore and its small canals; *g*, unicellular gland.
Mag. 335.
5. Female tail seen sideways: *g*, rectal glands; *a* anus; *p*, lateral papillae. Mag. 335.
6. A, B. Male tail seen fullface and sideways: *i*, intestine; *c*, deferent canal; *g*, rectal gland.
Mag. 395.
- Fig. 7. Spicule and gubernaculum (accessory piece) seen sideways. Mag. 1460.
8. Spermatozoa. Mag. 1460.
9. Eggs. Mag. 335.
10. Adult female of *Rhabditis Vigueri*: *v*, vulva; *g*, ovary; *a*, anus. Mag. 150.
11. Anterior end, mouth and buccal cavity. Mag. 1280.
12. Esophagus: *c*, nerve ring; *p*, excretory pore. Mag. 390.
13. A, B. Male tail, bursa, papillae and spicules, seen fullface and sideways. Mag. 705.
14. Eggs of *Rhabditis dolichura* at the two- and four-blastomere stage. Mag. 335.
15. Spermatozoa of the same. Mag. 1510.

PLATE XXI

- Fig. 1. Adult female of *Rhabditis dolichura*: *v*, vulva; *g*, ovary; *a*, anus. Mag. 150.
2. Anterior extremity, mouth and buccal cavity. Mag. 830.
3. Esophagus: *c*, nerve ring; *p*, excretory pore with its small canals. Mag. 395.
4. Female tail: *r*, rectum with its glands; *a*, anus. Mag. 395.
5. Anterior part of the intestine to show the arrangement of its nuclei and of its cells. Mag. 335.
6. Oviduct and seminal receptacle *ov*, vitellogen *vi*, and germigen *g*. Mag. 705.
7. A, B. Two genital organs at the time of their arrival at maturity. The first, A, beginning to produce the first spermatozoa; the second, B, having produced all its spermatozoa and maturing its first ova. Max. 705.
8. A, B. Tail, bursa and male spicules seen fullface and sideways: *i*, intestine; *c*, deferent canal; *g*, rectal glands. Mag. 705.
9. Female genital organ of *Rhabditis coronata*: *v*, vulva; *u*, uterus; *r*, seminal receptacle; *ov*, ovary; *i*, intestine. Mag. 800.
10. Egg of the same. Mag. 705.
11. Male tail of the same seen sideways; with its bursa, its papillae and its spicules. Mag. 705.

PLATE XXII

- Fig. 1. Adult female of *Rhabditis coronata*: *v*, vulva and genital organs; *a*, anus. Mag. 335.
2. Anterior end, mouth and buccal cavity. Mag. 800.
3. Esophagus: *c*, nerve ring; *p*, excretory pore. Mag. 705.
4. Adult female of *Diplogaster robustus*: *v*, vulva; *o*, oviduct; *g*, ovary; *a*, anus. Mag. 84.
5. Vein and striations of the cuticle. Mag. 1460.
6. Anterior end, mouth and buccal cavity. Mag. 800.

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7. Esophagus: *c*, nerve ring; *p*, excretory pore and organ. Mag. 335.
8. Female tail: *g*, rectum and rectal glands; *a*, anus; *p*, lateral papilla. Mag. 335.
9. Genital organ of a young female: *v*, vulva and vagina; *u*, uterus still containing only two eggs; *ss*, spermatozoa; *o*, oviduct; *g*, ovary, in the front the vitellogen, in the rear the germigen. Mag. 188.
10. Eggs at the two- and four-blastomere stage. Mag. 335.

Fig. 11. Rhaphides of the testis. Mag. 1460.

12. Spermatozoa of male and female origin. Mag. 1460.
13. Spermatic nuclei treated with acetic acid and showing their twelve chromosomes. Mag. 1460.

PLATE XXIII

- Fig. 1. Male *Diplogaster robustus* seen sideways with its papillae Mag. its spicu 335.
2. Spicules and gubernaculum (accessory piece) seen sideways. Mag. 705.
 3. Juvenile having just hatched: *r*, genital rudiment; *p*, excretory pore and nerve ring; *a*, anus. Mag. 335.
 4. Anterior end, mouth and buccal cavity of the previous. Mag. 1460.
 5. Genital rudiment of the same. Mag. 1460.
 6. Adult female of *Diplogaster minor*: *v*, vulva and genital organs; *a*, anus. Mag. 150.
 7. Anterior end, mouth and buccal cavity. Mag. 800.
 8. Esophagus: *c*, nerve ring; *p*, excretory pore. Mag. 335.
 9. Female tail: *g*, rectum and rectal glands; *a*, anus. Mag. 335.
 10. Genital organs : *v*, vulva; *u*, uterus; *o*, oviduct (tuba); *g*, ovary. Mag. 150.
 11. Egg at the two-blastomere stage. Mag. 335.
 12. Parthenogenetic egg with a misshaped shell. Mag. 335.
 13. Parthenogenetic egg of regular shape and content in the morula state. Mag. 335.
 14. Anterior end, mouth and buccal cavity of *Cephalobus dubius*, race *rotundata*. Mag. 1510.
 15. Anterior end, mouth and buccal cavity of the *Cephalobus dubius*, race *apicata*. Mag. 1510.
 16. Tail of race *apicata*. Mag. 705.

PLATE XXIV

- Fig. 1. Adult female of *Cephalobus dubius*: *v*, vulva; *u*, uterus; *g*, ovary; *a*, anus; *p*, excretory pore and nerve ring. Mag. 335.
2. Tail of race *rotundata*. Mag. 705.
 3. Adult female of *Cephalobus lentus*: *v*, vulva; *u*, uterus; *g*, ovary; *a*, anus. Mag. 150.
 4. Anterior end, mouth and buccal cavity. Mag. 1510.
 5. Esophagus: *c*, nerve ring; *p*, excretory pore and small canal. Mag. 705.
 6. Tail: *m*, lateral membrane; *r*, rectum and rectal glands; *a*, anus. Mag. 380.

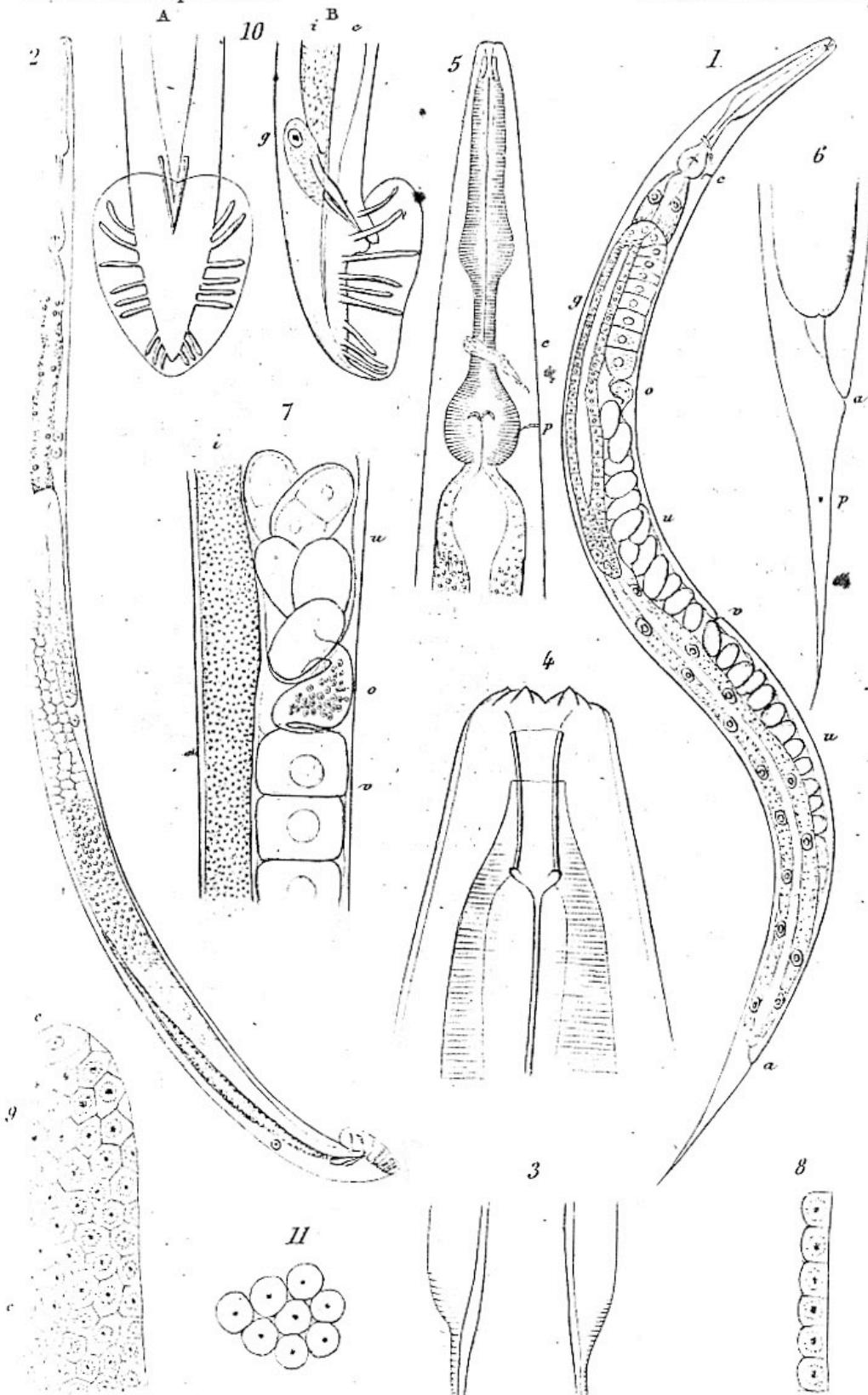
7. Eggs: Mag. 335.
8. Juvenile having just hatched: *g*, genital rudiment; *a*, anus. Mag. 335.
9. Anterior end, mouth and buccal cavity of the same. Mag. 1510.

PLATE XXV

- Fig. 1. Genital organ of *Plectus cirratus*: *v*, vulva and vagina; *uu*, the two uteri each containing five eggs; *cc*, oviducts with the cells showing the pseudo-spermatozoa; *o*, ovary; *i*, intestine. Mag. 335.
2. Adult female of *Aphelenchus agricola*; *v*, vulva; *u*, uterus; *g*, ovary; *a*, anus. Mag. 75.
 3. Anterior end, mouth and stylet. Mag. 1510.
 4. Esophagus: *c*, nerve ring; *p*, pore and excretory canal; *e*, posterior limit of the esophagus; *i*, narrow part of the intestine. Mag. 705.
 5. Tail: *m*, lateral membrane; *r*, rectum; *a*, anus. Mag. 705.
 6. Genital organ: *v*, vulva; *u*, uterus; *o*, ovary. Mag. 335.
 7. Egg at the three-blastomere stage. Mag. 335.
 8. Anterior end and mouth of *Alaimus Thamugadi*. Mag. 1460.
 9. Tail of the same: *d*, intestinal diverticulum. Mag. 335.

PLATE XXVI

- Fig. 1. Adult female of *Alaimus Thamugadi*: *v*, vulva and genital organ; *a*, anus. Mag. 150.
2. Esophagus: *c*, nerve ring. Mag. 335.
 3. Egg at the four-blastomere stage. Mag. 335.
 4. Adult female of *Macrolaimus crucis*: *v*, vulva; *u*, uterus; *g*, ovary; *a*, anus. Mag. 150.
 5. Anterior end, mouth and buccal cavity. Mag. 1510.
 6. Other anterior end, with lateral tooth in the buccal cavity. Mag. 1510.
 7. Esophagus: *c*, nerve ring; *p*, excretory pore and organ. Mag. 105.
 8. Tail: *r*, rectum and rectal glands; *a*, anus. Mag. 705.
 9. Genital organ: *v*, vulva; *u*, uterus; *o*, oviduct (tuba); *g*, ovary. Mag. 335.
 10. Eggs. Mag. 335.

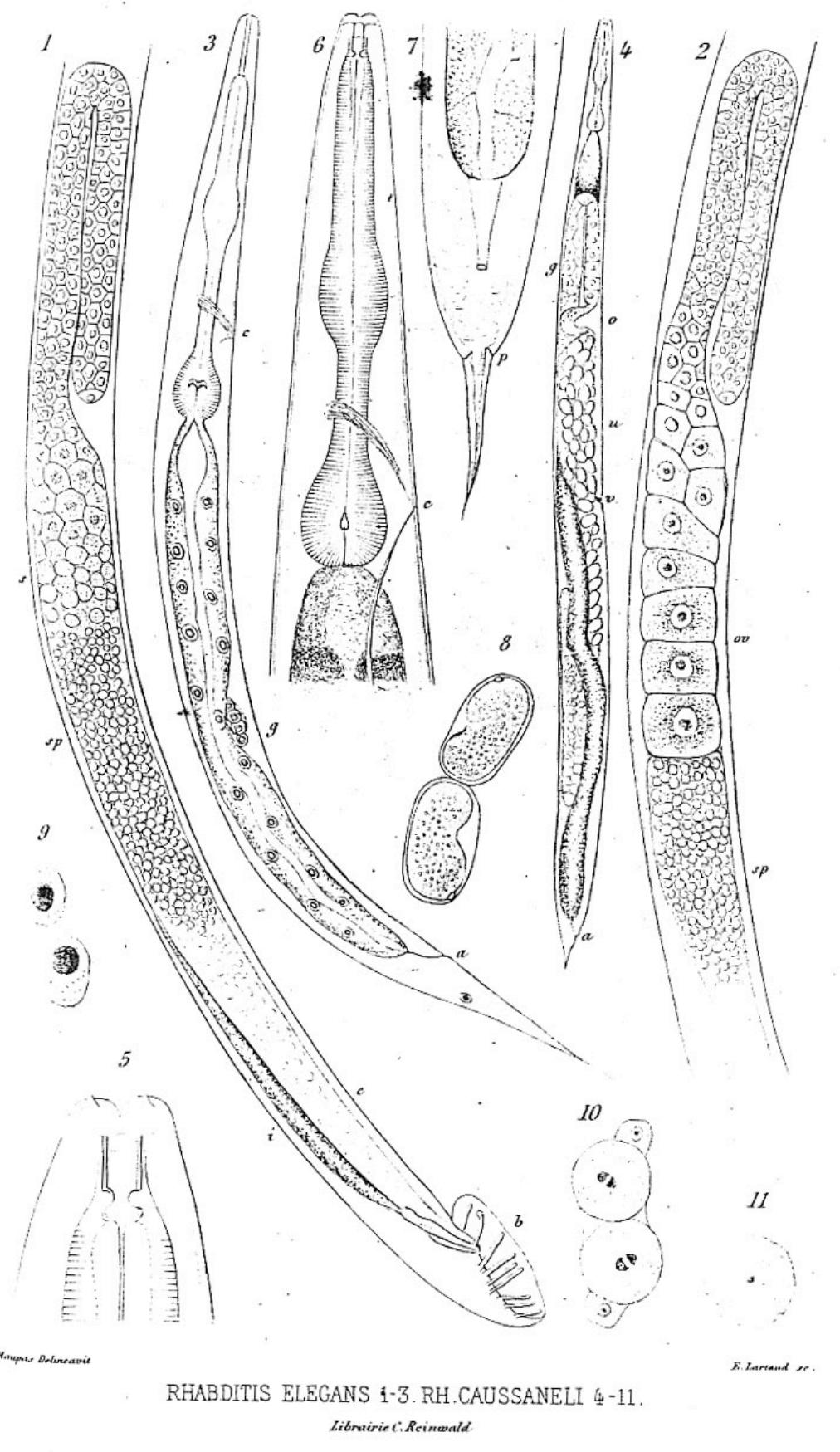


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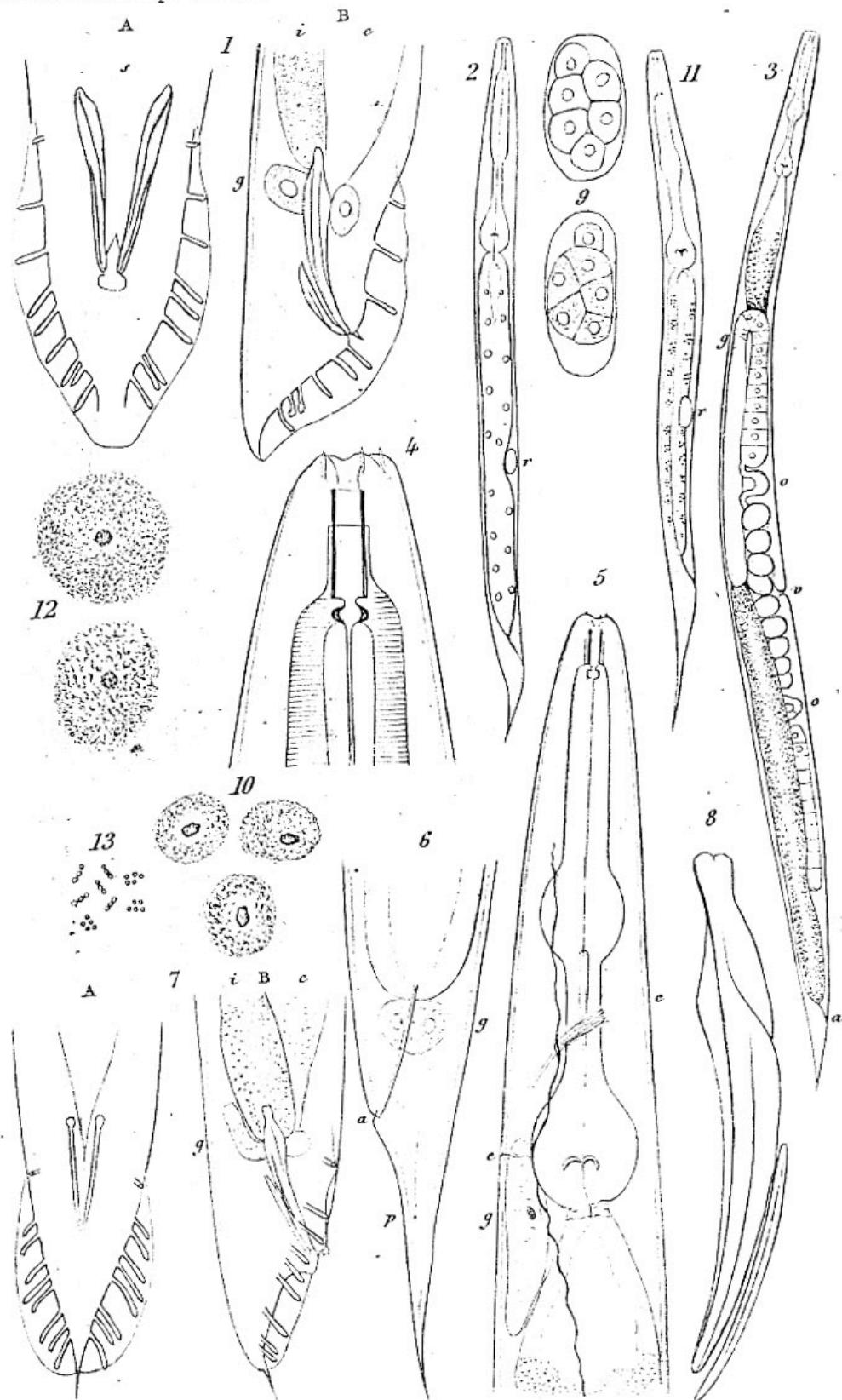


RHBADITIS ELEGANS 1-3. RH. CAUSSANELLI 4-11.

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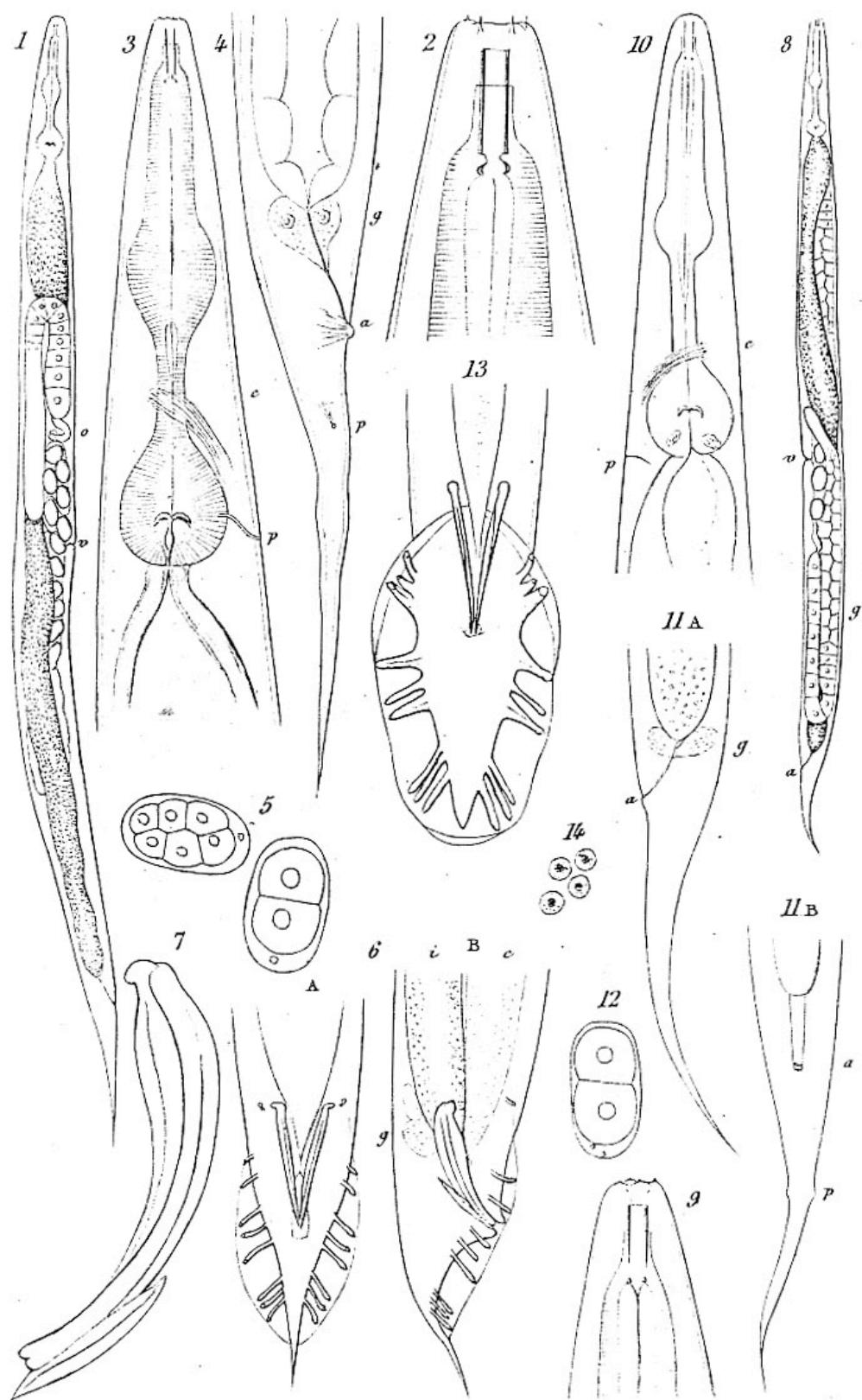


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RHABDITIS CAUSSANELI 1-2. RH. MARIONIS 3-11. RH. DUTHIERSI 12-13.

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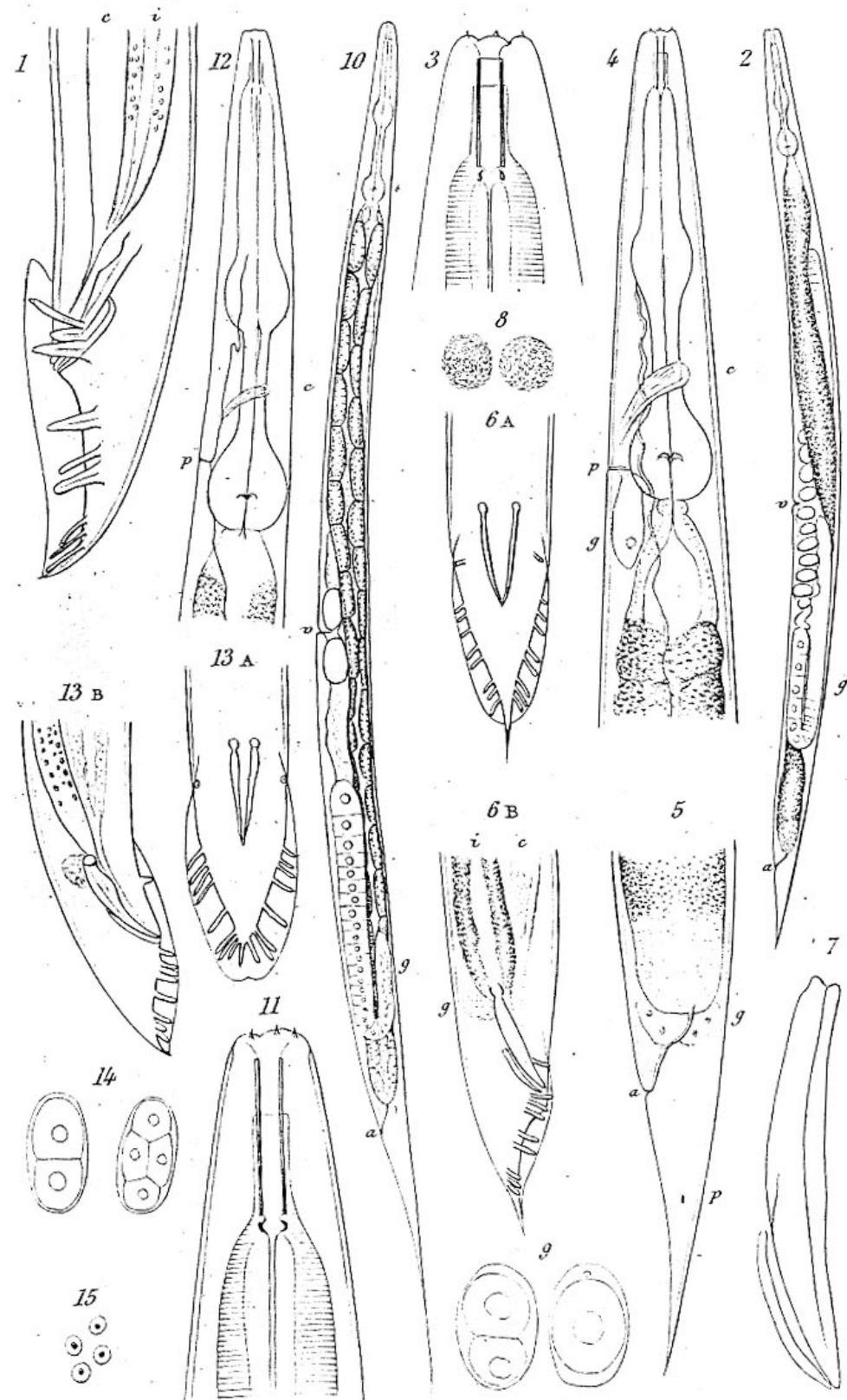


Naupus Dolinocoris

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RHABDITIS DUTHIERSI 1-7. RH. PERRIERI 8-14.

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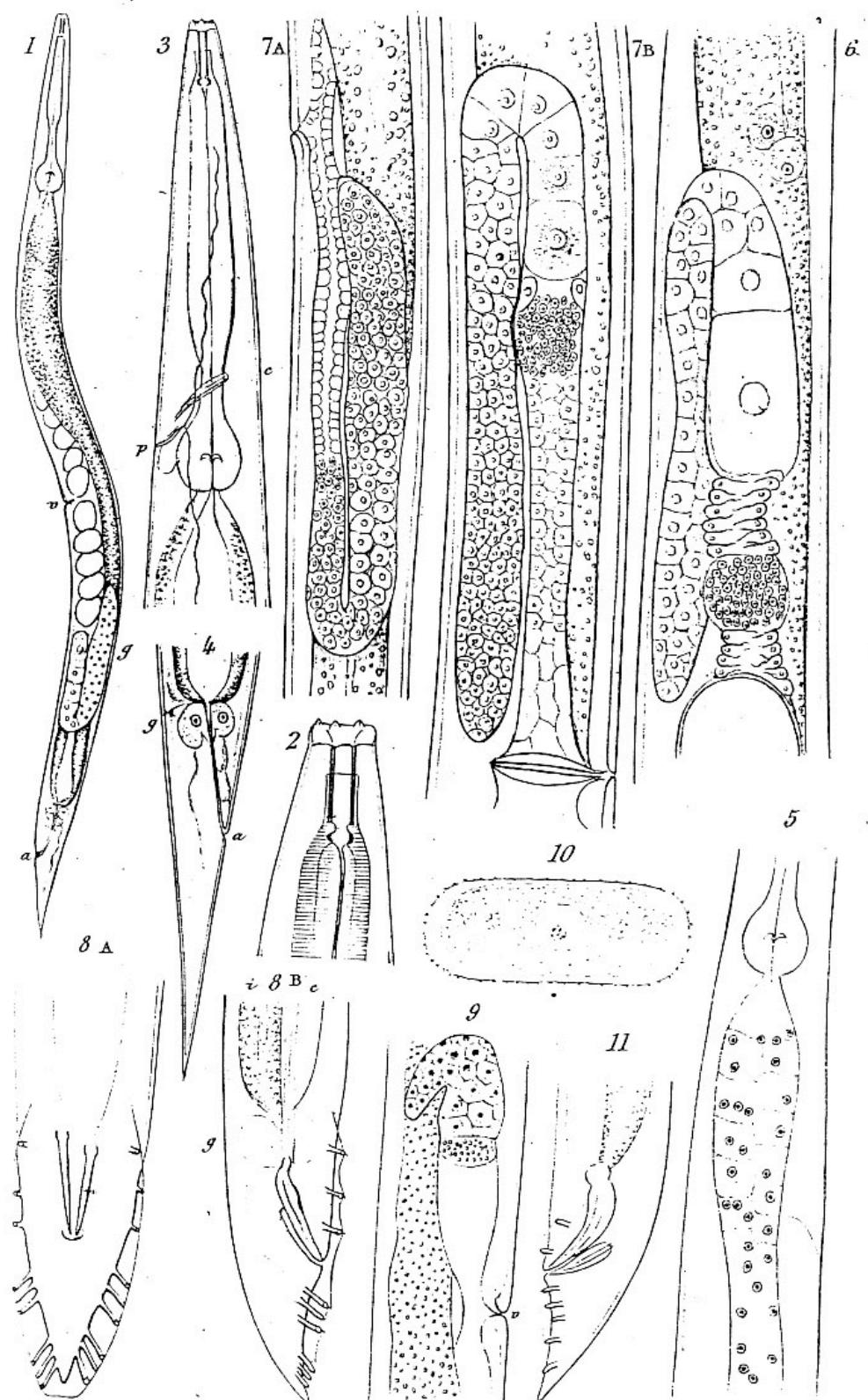


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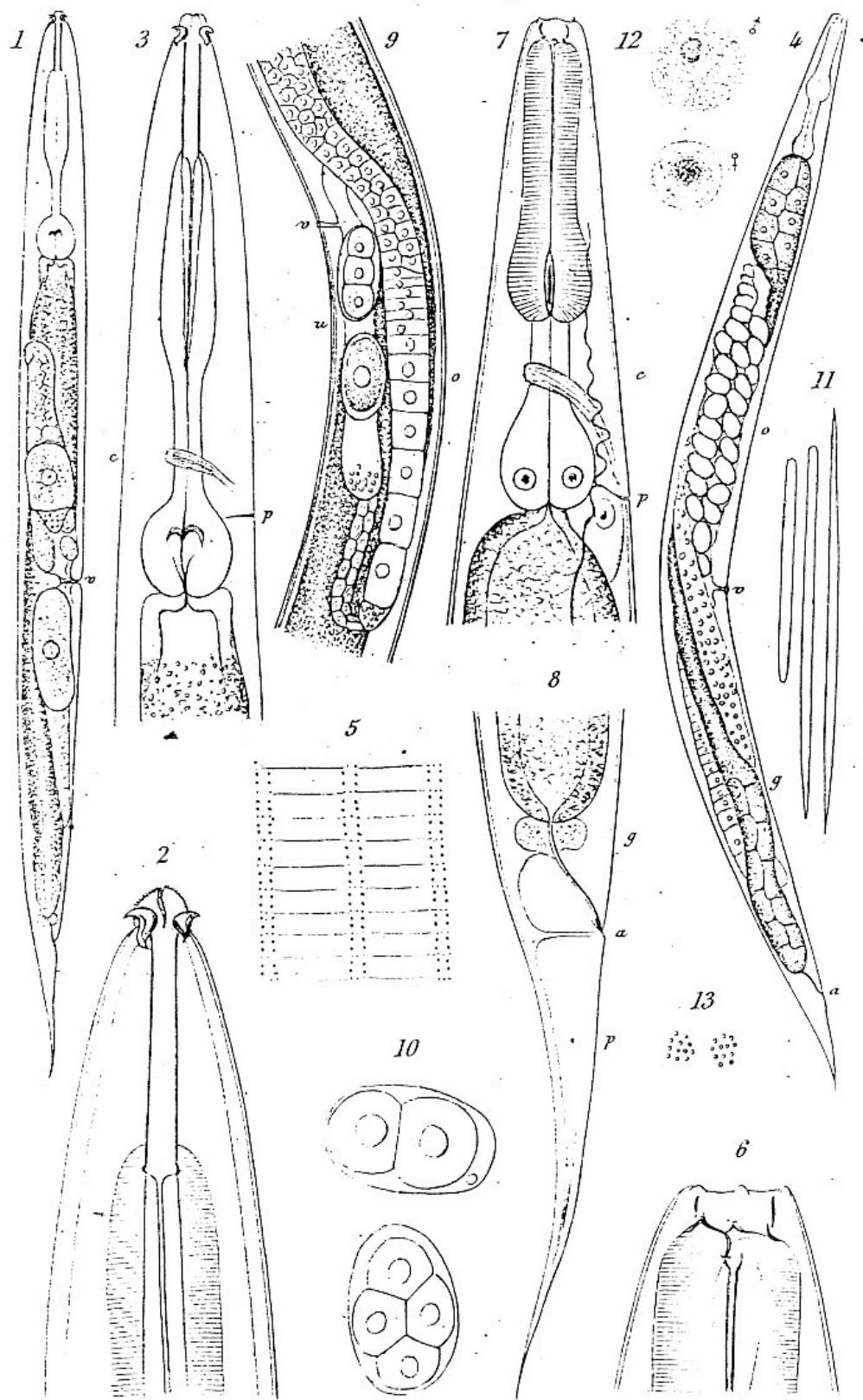
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RHABDITIS DOLICHURA 1-8. RH. CORONATA 9-11.

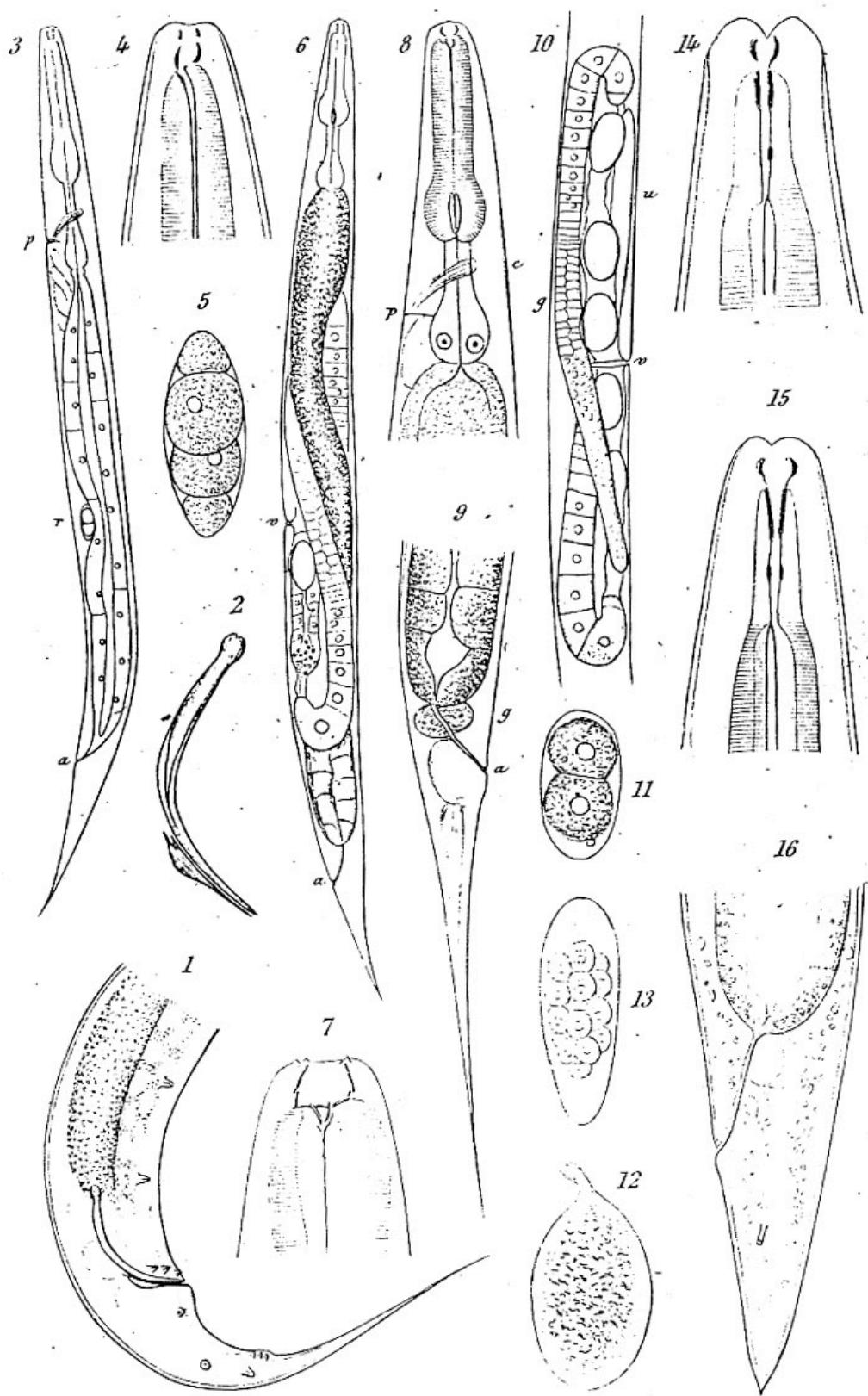


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RHABDITIS CORONATA 1-3 DIPLOGASTER ROBUSTUS 4-13

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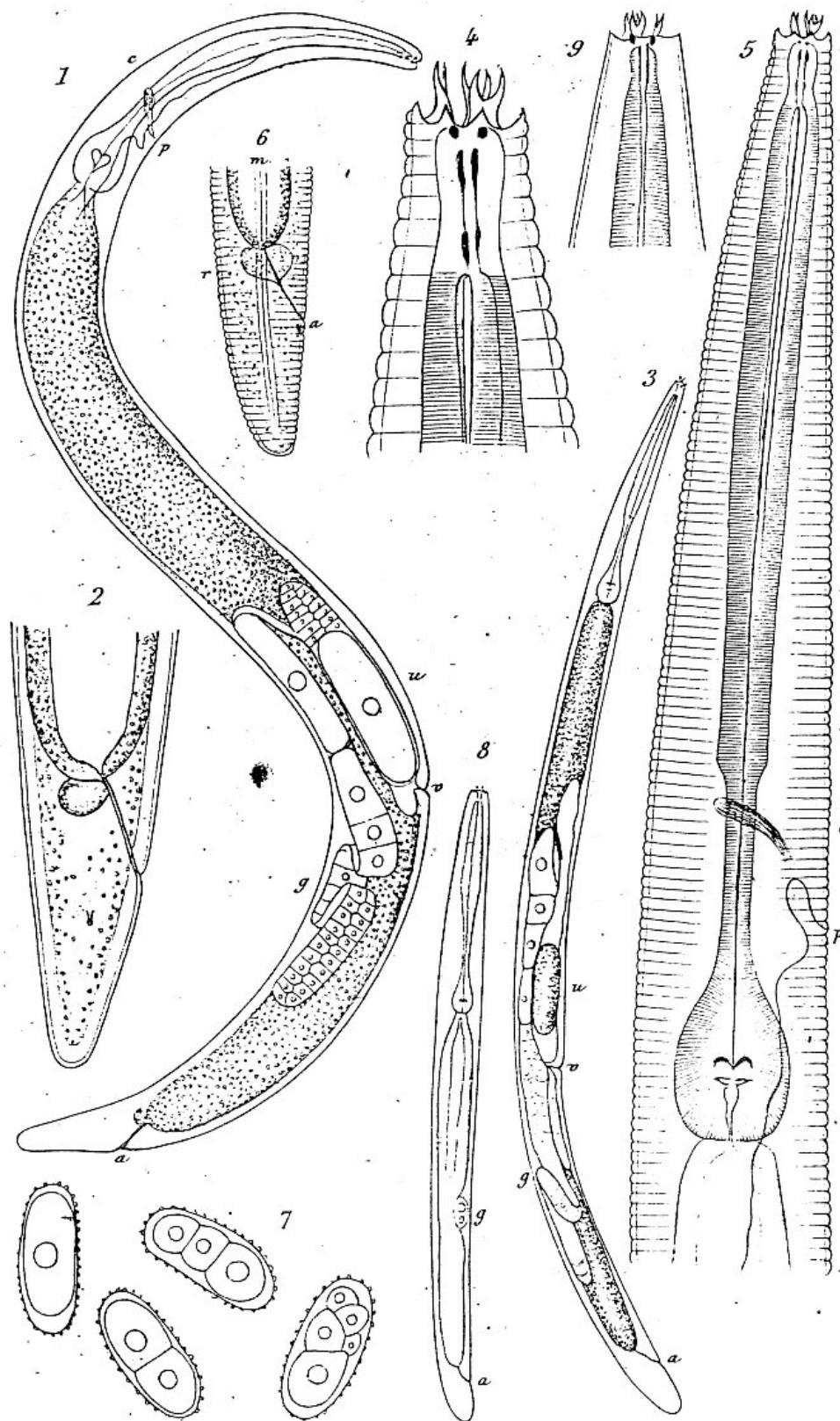
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DIPLOGASTER ROBUSTUS 1-5 DIP MINOR 6 13 CEPHALOBUS DUBIUS 14-16

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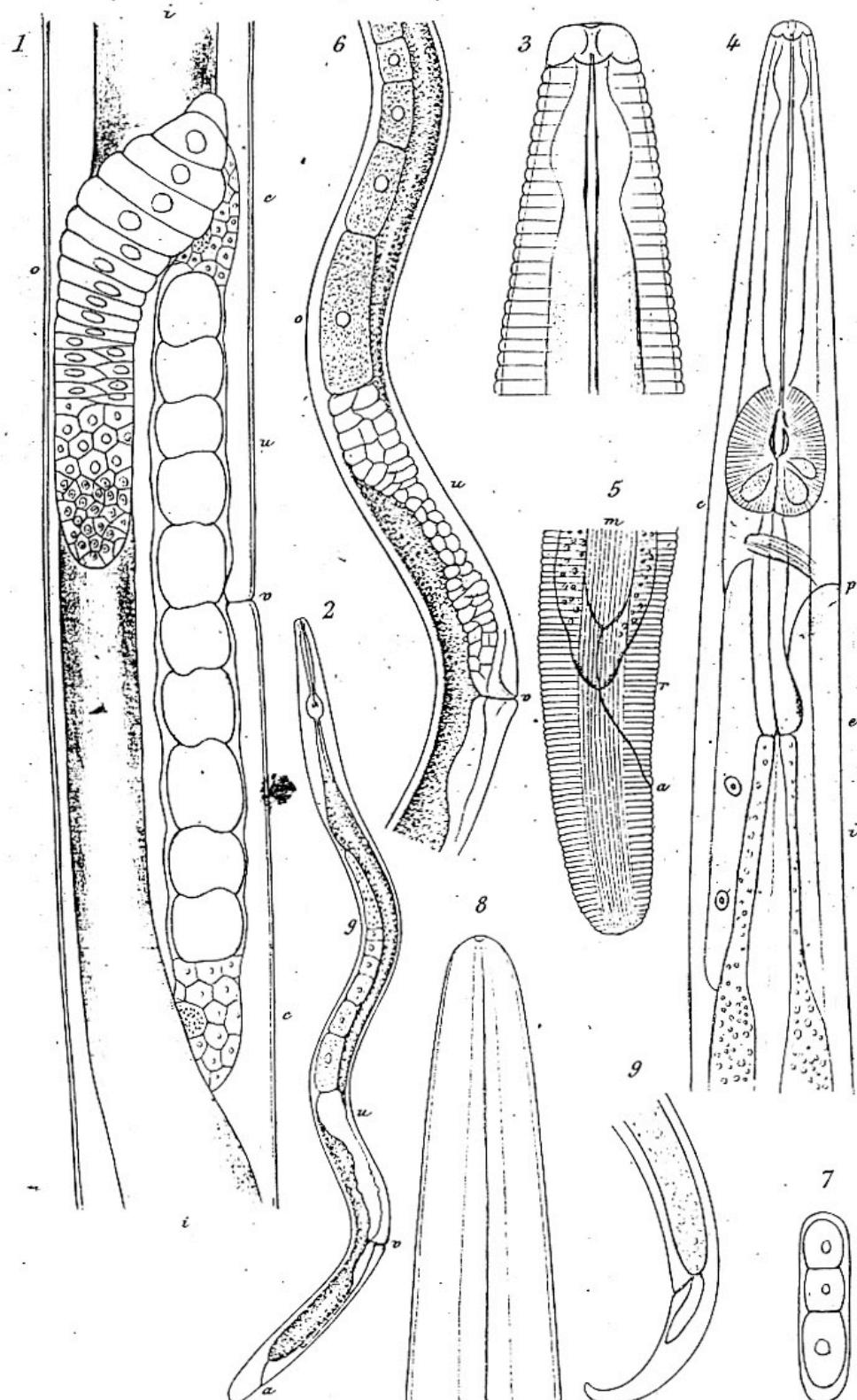


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CEPHALOBUS DUBIUS 1-2. CEPH. LENTUS 3-9.

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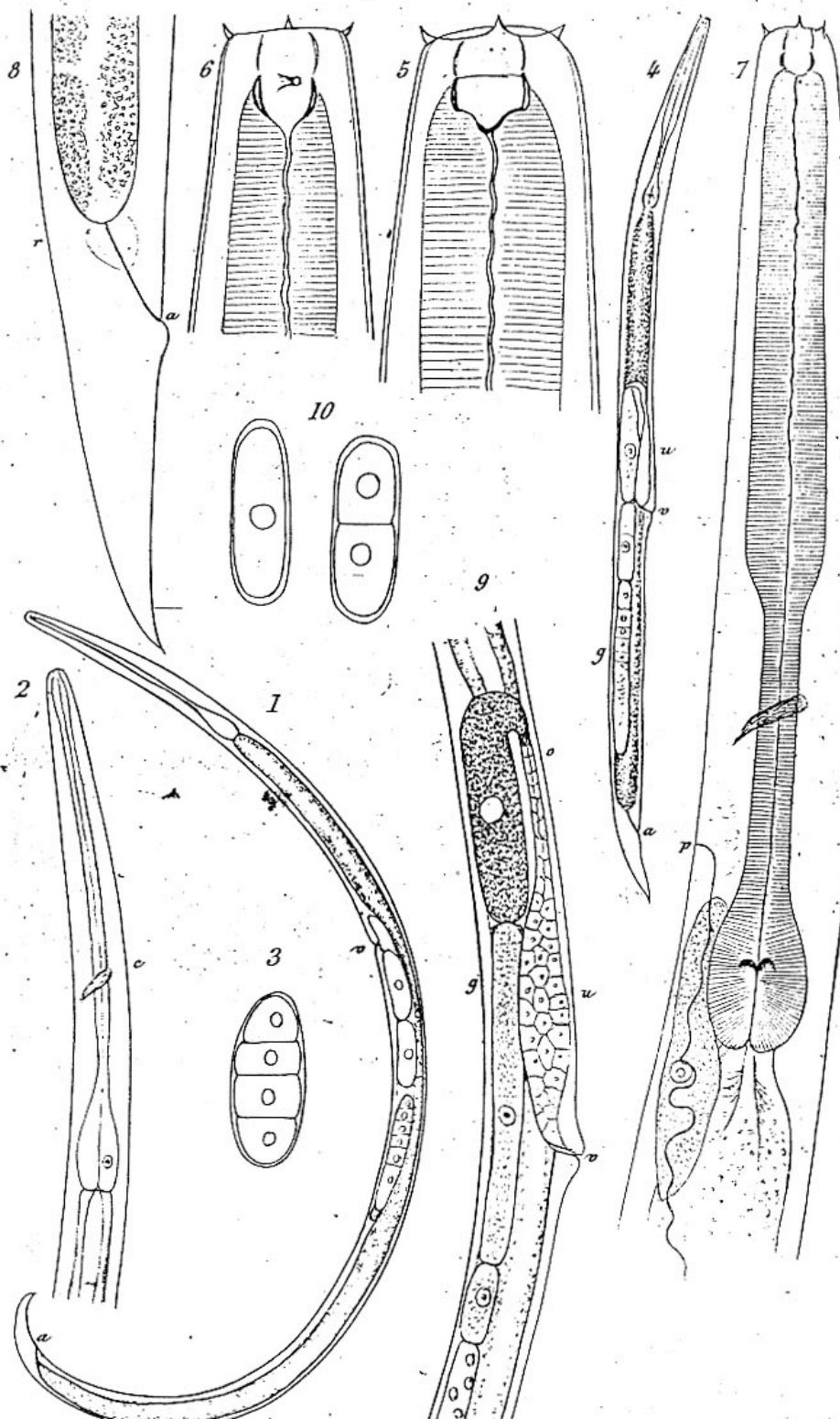
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PLECTUS CIRRATUS 1. APHELENCHUS AGRICOLA 2-7. ALAIMUS THAMUGADI 8-9.

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ALAIMUS THAMUGADI 1-3. MACROLAIMUS CRUCIS 4-10.

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