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Re-Claiming Adrien Certes (1835-1903), Pioneer Deep-Sea Microbiologist, as a Protistologist

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Abstract

Since the advent of deep-sea microbiology in the 1950's, microbiologists have justifiably recognized Adrien Certes as a pioneer due to his early studies on culturing deep-sea bacteria, and investigating the effects of pressure on microorganisms. However, Certes was actually first and foremost a protistologist. He was but a 'part-time' scientist, without advanced training in biology, and only began publishing in middle age. His 'day job' was a high-level civil servant, an *Inspecteur des Finances*. Nonetheless, he authored over 50 works on a remarkably wide range of free-living and commensal protists as well as microscopical methods. He interacted with major figures of biology in France of his time such as the protistologist Édouard-Gérard Balbiani, the microbiologist Louis Pasteur, and the zoologist Alphonse Milne Edwards, although exactly how his connections were forged unfortunately remains obscure. Certes, acknowledged by marine microbiologists, is largely overlooked in accounts of protistology, perhaps because his work was dispersed over a variety of topics and taxa. Also, as a part-time researcher, he left no academic children behind. Here the life and scientific work of Certes are reviewed and it is shown that he was a devoted protistologist.

Keywords: History of Protistology, Ciliophora, Endocommensals, Fixation, Staining

Introduction

Adrian Certes is a name relatively well known to those familiar with the history of marine microbiology as a pioneer in deep-sea microbiology (*e.g.*, Karl & Proctor 2007). He is credited with the first cultures of bacteria from deep-sea sediments and studies of pressure tolerance of microorganisms (Adler & Dücker 2017), which he carried out in the laboratory of Louis Pasteur and published in 1884 (Suppl File Refs 27-31). Certes showed that bacteria were present in the deep-sea sediments and capable of metabolism. Much lesser known is the fact that he was first and foremost a protistologist, not a bacteriologist. The famous deep-sea microbiology studies were not his first. His first 2 scientific publications in 1879 were on the use of osmic acid as a new method for preserving 'infusoria' and the third on an opalinid, a frog endosymbiont, and the taxonomy of opalinids (Suppl File Refs 3-5). The samples from which Certes cultivated deep-sea bacteria had been collected for him by Alphonse Milne Edwards, one of the organizers of the deep-sea explorations of the *Travailleur* and *Talisman* (1880-1883) in the hopes of finding deep-sea protists. Certes had been named as responsible for 'infusoria and other protozoa' (Milne Edwards 1881). Before his deep-sea

bacteria studies, Certes had reported on his surprise and disappointment in not finding ciliates or flagellates in deep-sea sediments (Suppl File Refs 16,18). While protistology was Certes' specialty, not bacteriology, he is largely overlooked in histories of protistology. For example Cole (1926) makes no mention of Certes and Corliss (1978) mentions Certes only in passing. This is despite the fact that he made many contributions concerning a wide variety of protistan taxa and microscopical methods. Here the life and scientific contributions of Adrien Certes are reviewed and some speculations as to the reasons for his relative obscurity are provided.

Biographical Sketch

Adrien Certes (how he signed his publications) was born in Paris in 1835 as Marie Louise Aldolphe Adrien Certes. His father was a civil servant; the family was solidly middle class. He attended law school, graduating with a "Licence" (equivalent to a bachelor's degree) in Law in 1857. Rather than pursuing law, he began working in the Ministry of Finance as a contractual employee. In 1860, he passed the civil service examination beginning his career in a branch of the Ministry of Finance, 'Inspecteur Générale des Finances', as an assistant. Certes moved through the ranks (4th, 3rd, 2nd, 1st class) and was promoted to the top rank, *Inspecteur Générale des Finances* in 1886. Certes was awarded the order of Knight in the Legion of Honor in 1877, the year he was promoted to 1st class, and promoted to Major in the Legion of Honor in 1887, just after being named *Inspecteur Générale des Finances*. His service to Ministry of Finance was specifically cited in both of his Legion of Honor nominations (Knight and Major). It should be noted that the corps of *Inspecteur Générale des Finances* is one of the most prestigious positions within the French civil service, long considered an elite in France (e.g. Piétri 1962), the alumni of which in recent years include a Prime Minister (Alain Juppé) and a past (Valéry Giscard d'Estaing) as well the present President of the Republic (Emmanuel Macron). Adrien Certes was married in 1865 to Marie Depaul and they had a daughter, Marie Madeleine in 1872. Certes died rather suddenly of an unnamed 'grave disease' in 1903, at the age of 68, just 3 years after retirement. The portraits in Fig. 1 show Certes at the age of 31, just after marriage and at the age of 53, an established scientist.



Fig. 1. Left panel: Portrait of Adrien Certes at the age of 31, when he was Inspecteur des Finance 3rd class (1866), 13 years before the appearance of his first scientific publication. Right panel: Certes at the age of 53 (1888), *Inspecteur Générale des Finances*, a former President of the Société Zoologique de France, and at that time already author of over 30 scientific papers.

The Enigma of Certes the Biologist

From what is documented in the public record, Certes appears to have had neither an advanced training nor a particular interest in biology until middle age. Obviously, one cannot exclude the possibility that he had always been an avid amateur naturalist. However, the first public instance of an interest in biology appeared in a long report entitled "*Phylloxera et le Budget*" he authored in 1877 (at the age of 42) on the vineyard insect pest *Phylloxera* that devastated the French wine industry in 1860's and 1870's. As the title suggests, the report was largely concerned with the financial aspects effecting both vintners and the state, of the near collapse of the wine industry (Suppl File Ref 1,2). He concluded the report with financial arguments for establishing an insurance fund to protect the wine industry from disasters. However, a large part, nearly 50% of the report, was devoted to the natural history of the insect whose complex life-cycle, with an alternation of generations, had been revealed by Édouard-Gérard Balbiani, the developmental biologist and protistologist. He quoted at length from Balbiani's previous work and from an Academy of Science report authored by, among others, Louis Pasteur and Alphonse Milne Edwards. It is reasonable to suppose that in researching *Phylloxera*, Certes may have contacted them. He may have known them beforehand. Unfortunately there are no records available. Of the four men, only Pasteur's correspondence has been preserved and documented; no mention of Certes appears among Pasteur's letters or laboratory notebooks. In any event, Balbiani, Pasteur and Milne Edwards all appear to have played a significant role in Certes' life as a biologist.

Balbani, recognized as the first observer of conjugation in ciliates (Corliss 1978), would be called 'my friend and master' by Certes in later years (Suppl File Ref 21, 54) and Certes would eventually furnish Balbiani with sketches of rumen protists for his lectures at the *Collège de France* (Suppl File Ref 37). As mentioned previously, Milne Edwards, in organizing the deep-sea explorations of the

Travailleur and *Talisman*, would soon name Certes as the expedition expert on "Infusoria and other protozoa" and collect deep-sea samples for him (Milne Edwards 1881). Certes' studies of bacteria, cultured from deep-sea sediments, and the effects of pressure on bacteria, protists, and yeasts, would be carried out in Pasteur's laboratory, as Certes noted in his reports (Suppl Refs 27-31). Much of Certes' work would be formally presented to the Academy of Sciences, through a member of the Academy, as was the custom, first by Pasteur (Suppl File Refs 3,6,7) and later by Milne Edwards (Suppl. File Refs 14, 20, 27, 46). Certes would eventually name a new oyster endo-commensal flagellate species for Balbiani, *Cristispira balbiani* (Suppl File Ref 21) now known as a prokaryote, and a planktonic phaeodarian *Challengeron edwardsi* for Milne Edwards (Suppl File Ref 4).

The entrance of Certes into the biological community in France occurred shortly after the presentation to the Academy of Sciences, by Pasteur, of Certes' protocol for fixing protists with osmic acid. Notably, unlike his reports on deep-sea cultures and pressure effects, the osmic acid reports of Certes and Certes' subsequent reports on techniques of vital staining, were not said to have been carried out in Pasteur's laboratory. Where Certes worked before and after working in Pasteur's laboratory is not known but his early work quickly gained him some recognition. Pasteur presented his paper in March of 1879 (Suppl File Ref 3). In May of 1879 Certes published a longer version of the paper with a color plate (Fig. 2) in the *Journal de Micrographie* (Suppl File Ref 4) and in the same month was nominated to the *Société Zoologique de France*. Although the society was young (only 4 years old) it already had a membership of nearly 200. By December of 1879 he began publishing in the *Bulletin de la Société Zoologique de France*, with a paper on an opalinid commensal of a frog (Suppl File Ref 5) the first of 20 papers that he would eventually publish in the society's journal.

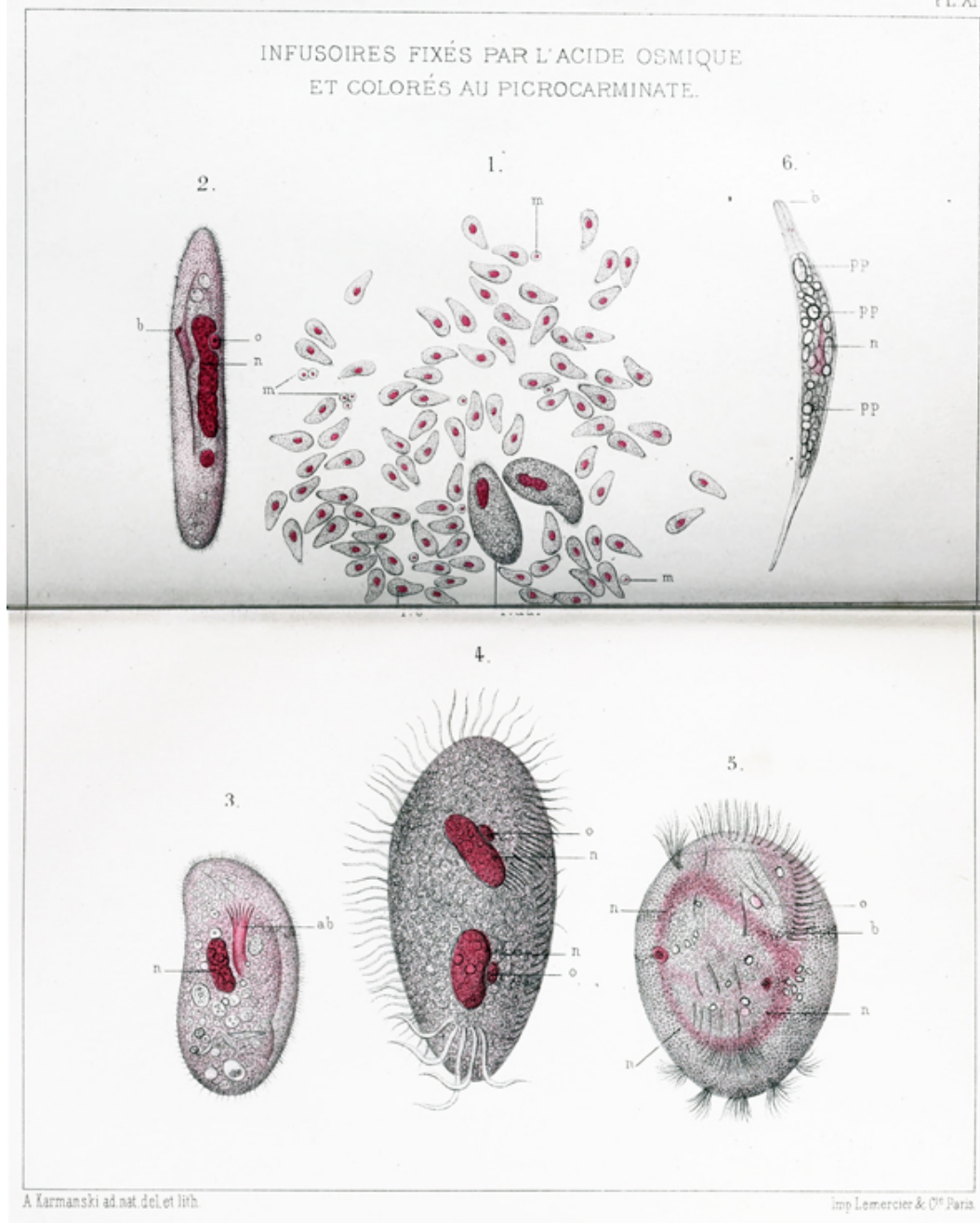


Fig. 2. "Infusoria fixed with osmic acid and stained with picocarminate" from the 1879 publication in the *Journal de Micrographie* (Suppl File Ref 4). The fold-out plate was published without a legend in the two different digital versions available (<https://www.biodiversitylibrary.org/bibliography/79063#/details>).

The *Société Zoologique* was the primary scientific home of Certes. He was elected President in 1887 and remained an active member of the board of directors until his death in 1903. His widow carried on a Certes membership in the society until her death in 1917. An obituary notice of Certes (Guiart 1903) recorded that, although he was *Inspecteur Générale des Finances* and a Major in the Legion of

Honour, the title of which he was most proud of was that of Former President of the *Société Zoologique*.

The Scientific Work of Adrien Certes

Certes published in the scientific press from 1879 (at the age of 44) until his death in 1903 (at the age of 68). Certes had then a relatively short public scientific career of 24 years. However there is evidence that he was working on biological questions in 1878. One of his major interests was encystment and excystment of both marine and freshwater protists and other microorganisms. One of his early publications was on the resting stages of the brine shrimp *Artemia* and the ciliate *Blepharisma* (Suppl File Ref 14) in which he states that he collected sediment samples from an Algerian saltern in 1878. His last publication appeared in 1903, the year of his death. It was an extensive review of resting stages and cyst studies (Suppl File Ref 54). In it he states that his laboratory notes go back 25 years, bringing us back again to 1878 as the start of his scientific studies. By 1883 he had published a pamphlet on techniques for the microscopic analysis of natural waters (Suppl File Ref 25) focusing largely on protists (see Figure 3) and it was favorably reviewed (Suppl File, Further Reading).

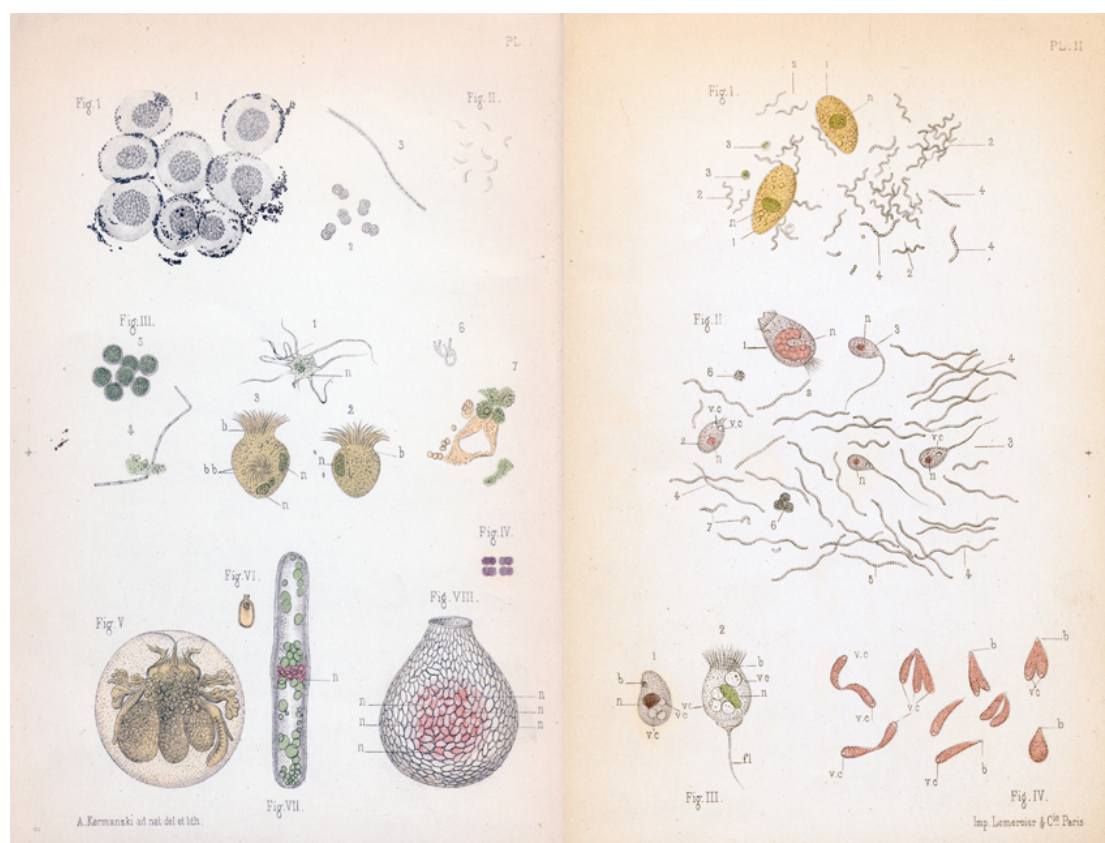


Fig. 3. Plates I and II from Certes 1883 *Analyses Micrographique des Eaux* showing microorganisms from a variety of natural waters and a hay infusion stained using a variety of colorants after fixation with osmic acid or heat shock. The oligotrich in the center of Plate 1 shown in early stomatogenesis is apparently the first depiction of an oligotrich undergoing cell division (Sabine Agatha, personal communication).

While short, the scientific career of Certes was highly productive, especially considering that science was a part-time occupation for him. He authored 52 scientific publications. Figure 4 shows the journals in which most of his work appeared and the topics on which he most frequently published. He published the most articles in the *Bulletin de la Société Zoologique de France* on a wide range of topics. His first paper in the journal was on a frog opalinid (Suppl File Ref 5) and the last on sampling and preserving sediment samples (Suppl File Ref 51). Overall, he reported most frequently on microscopical methods addressing topics such as fixation, post-fixation and vital staining, enumeration, and microscope optics. The methods papers largely concerned protists as target taxa (Suppl File Ref 3,4,7- 12,15,22,24,25,23,33,42) with but two focused on prokaryotes alone (Suppl File Ref 49,53).

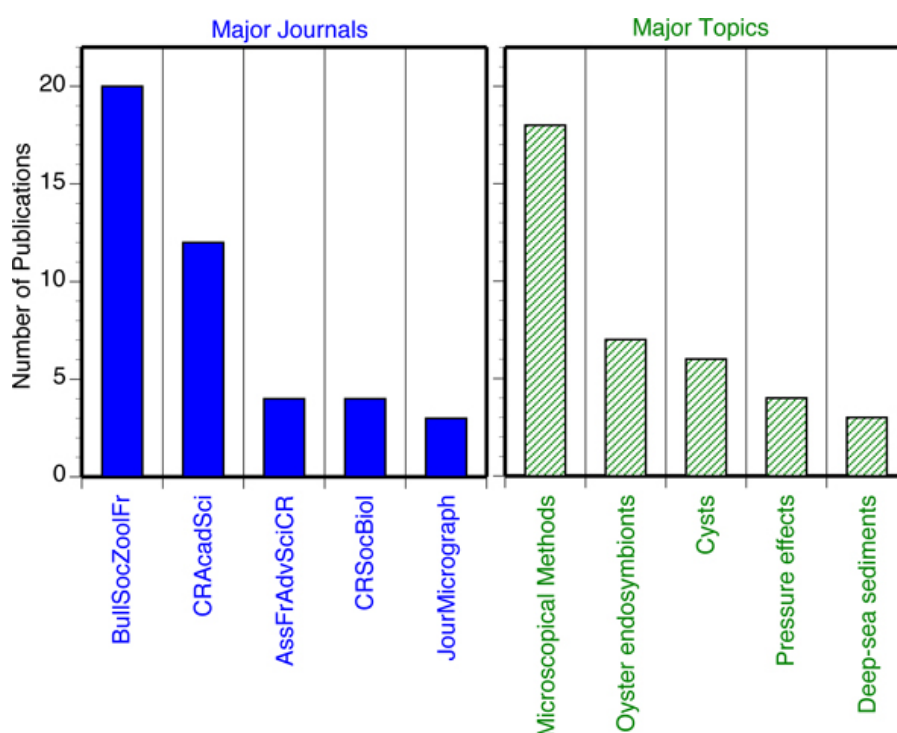


Fig. 4. The major journals and topics of the scientific production of Adrien Certes. The left panel shows the journals in which Certes published 3 or more papers: *Bulletin de la Société Zoologique de France* (BullSocZoolFr), *Comptes rendus hebdomadaires des séances de l'Académie des Sciences* (CRAcadSci) *Association pour l'Avancement de La Science de France, Comptes Rendus* (AssFrAdvSciCR), *Comptes Rendus de la Société de Biologie* (CRSocBiol), and the *Journal de Micrographie*. The right panel show the number of papers he published by broad topic, limited to topics on which he published at least 3 papers. See the Supplemental File for bibliographic details and article topic classifications.

During his career, Certes described 21 organisms as new species, 18 of which were described as protist taxa (see Supp File, Certes spp). Of the 21, 10 are currently recognized as valid first descriptions (Table 1). They include a variety of protist taxa: ciliates, testate amoeba and a radiolarian (Fig. 5). The percentage of species described by Certes still currently accepted is about 48% that is reasonably high for a biologist in the latter part of the nineteenth century. For

example, the "currently accepted percentage of total species descriptions" of Edouard Claparède, William Saville-Kent and Ernst Haeckel are, respectively 27%, 19% and 37%, based on listings of "all" and 'accepted' species in the WoRMS database (<http://www.marinespecies.org/index.php>). Thus, although a self-taught and part-time scientist, much of his work has stood the test of time.

Table 1. Species currently credited to Adrien Certes.

Taxon	Name as Described	Currently Accepted Name	Reference
Bacteria	<i>Trypanosoma balbiani</i>	<i>Cristispira balbiani</i> (Certes 1882) Gross 1910	Suppl File Ref 19
Bacteria	<i>Ancyromonas ruminantium</i>	<i>Selenomonas ruminantium</i> (Certes 1889) Wenyon 1926	Suppl File Ref 36
Ciliate	<i>Conchophthirius metchnikoffi</i>	<i>Phacodinium metchnikoffi</i> (Certes 1891) Kahl 1932	Suppl File Ref 45
Ciliate	<i>Odontochlamys gouraudi</i>	<i>Odontochlamys gouraudi</i> Certes 1891	Suppl File Ref 45
Testate Amoeba	<i>Nebula martiali</i>	<i>Certesella martiali</i> (Certes 1889) Loeblich & Tappan 1961	Suppl File Ref 40
Testate Amoeba	<i>Nebula collaris</i> variety <i>a, b</i>	<i>Certesella certsi</i> (Certes 1889) Loeblich & Tappan 1961	Suppl File Ref 40
Testate Amoeba	<i>Nebula vas</i>	<i>Apodera vas</i> (Certes 1891) Loeblich & Tappan 1961	Suppl File Ref 40
Testate Amoeba	<i>Trinema constricta</i>	<i>Corythion constricta</i> (Certes 1891) Jung 1942	Suppl File Ref 40
Radiolarian	<i>Hymeniastrum hyadesi</i>	<i>Hymeniastrum hyadesi</i> Certes 1891	Suppl File Ref 40
Nematode	<i>Euboastrichus guernei</i>	<i>Criconema guernei</i> (Certes 1889) Hofmänner & Menzel, 1914	Suppl File Ref 40

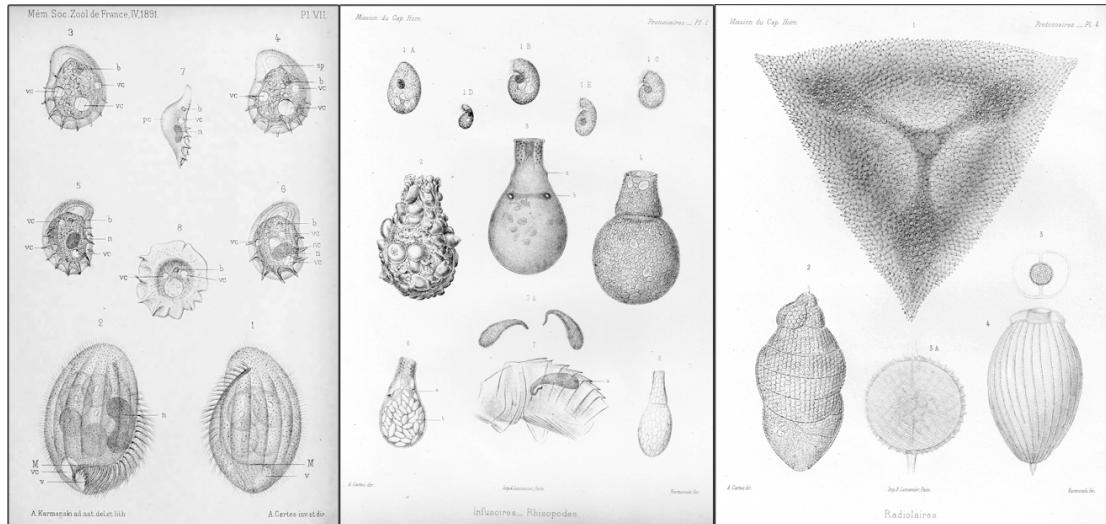


Fig. 5. The variety of new protist taxa described by Certes. The **left** panel shows the ciliates now known as *Phacodinium metchikoffi* (figs. 1,2) and *Odontochlamys gouraudi* (figs. 3-8) found examining frozen leaves from a Paris region forest in winter (Suppl File Ref 45). The **middle** panel shows the testate amoeba now known as *Certesella martiali* (Fig. 3), *Apodera vas* (Fig. 4, 5), and *Certasella certes* (Fig. 6) found in freshwater samples from the Cape Horn region (Suppl. File Ref 40); the genus *Certesella* was apparently named for Certes by Loeblich and Tappan (1961) but without any comments. The **right** panel shows the radiolarian *Hymeniasstrum hyadesi* (Fig. 1) from a deep-water sounding sample (7370 m depth) off the coast of Brazil (Suppl File Ref 40).

It can be said that Certes acted as a representative of protistology in both the community of French Zoologists and internationally. He participated in the annual meetings of the *Association pour l'Avancement des Sciences* talking on subject such as protistan endocommensals of oysters (Suppl. File Ref 21) and microscopical methods for the study of protists (Suppl. File Ref 23, 52). Certes was one of the organizers of the first International Congress of Zoology in 1889 (Blanchard 1990). The congress yielded the first version of the zoological nomenclature code. Among the congress participants, Certes and Géza Entz appeared to be the only representatives of protistology (Fig. 6). Certes would later participate in the fifth International Congress in Berlin in 1901 (Suppl. File Ref 53).



Fig. 6. The participants in the first International Congress of Zoology held in Paris in 1889. Certes and Géza Entz appear to be the only protistologists among the attendees (arrows). The formal charge of the Congress included formulation of a Zoological Code that would eventually be the International Code of Zoological Nomenclature. A first draft was published (Blanchard 1890) but was not unanimously adopted.

Why is Adrien Certes is Unknown to Protistologists?

Hopefully, here I have shown that Adrien Certes, while having made historic contributions to deep-sea bacteriology, was primarily a protistologist and a productive protistologist. His contributions to protistology were substantial in terms of the taxa he treated and the methodologies developed. He was the official "protistologist" for two major French expeditions: the *Travailleur* and *Talisman* deep-sea explorations (1880-1883) and the Cape Horn Expedition (1881-1883). One of the species he described from Cape Horn material, *Apodera vas*, has served as a prime example of a protistan taxon with a distinct biogeography (Smith & Wilkinson 2007). However, it would be disingenuous to ignore the fact that he did not make any fundamental contributions to the field. For example, he freely admitted that the microscopical methods he published on concerning fixation and staining were adaptations of new techniques in histology (Suppl File Ref 3,4). Certes, as a part-time scientist with an apparently private laboratory, was not involved in teaching or training activities and so produced no 'academic children'. These considerations likely explain the modern day anonymity of Certes except among deep-sea microbiologists. Nonetheless, Protistology can rightfully claim Adrien Certes.

Acknowledgments

Clément Duckert, Anna Kosakyan, and Nori Suzuki provided valuable information on the current status of species described by Certes. Amy Mantrone of the University of Chicago Library aided in the acquisition of Fig. 3, from a difficult to access publication. The comments and suggestions of the monitoring editor, Barry Leadbeater, and an anonymous reviewer led to significant improvements in the text. However, I retain full responsibility for all errors of fact, omission and interpretation.

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