(paraphrasing Martin Rees): 'absence of evidence is not evidence of absence'.

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http://dx.doi.org/10.1016/j.pt.2016.08.011

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Forum

Protist Collections: Essential for Future Research

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The vouchered deposit of protist type specimens in institution-maintained collections is a prerequisite for species description, and greatly enhances the chances of sample availability and preservation for future generations. However, specimens are currently most often deposited in personal collections maintained by the individual effort of researchers. We discuss the disadvantages of such a scenario and propose a change to this arrangement.

The sad truth is that, for historical reasons, protist collections are often relegated to dusty boxes of slides stored on shelves in an obscure corner of archives. However, for protozoologists, such collections have an invaluable interest because they often comprise reference type specimens deposited since the end of the 19th century. Interest in such collections has considerably decreased in the past few years as molecular data began to pave the road for new species (re)-description [1]. There is a general challenge to preserving scientific collections across the world. A deposit only in an individual laboratory collection greatly increases the likelihood that these specimens will be unavailable for research and reduces the chances of sample preservation for future generations. Some of these collections sometimes fail to survive, either by accidents or neglect. In 2010, a fire consumed more than 500 000 specimens of snakes, scorpions, and spiders, including several type specimens at the Butantan Institute in São Paulo, Brazil [2].

With the entry in the molecular era, natural history collections have evolved to meet the challenges of current and future interdisciplinary scientific studies. Many museums and research institutions developed new collections and information databases (DNA, tissue, culture, cryobanks, photographs, ethanol-fixed specimens, publication collections, and geographical and ecological information databases), which are of a first-rate importance, offering the opportunity to conduct integrative studies, including temporal and spatial surveys. The evolution of collections from static repositories to functional information systems is in response to increasing societal and scientific demands.

The well-known order Trypanosomatida contains the majority of catalogued species of the class Kinetoplastea, including public health-relevant species such as *Trypanosoma cruzi, Trypanosoma brucei*, and *Leishmania* spp. [3,4]. A few selected examples provided below illustrate the essential role of collections for current parasitology research.

Studies on ancient human remains changed the widely accepted theory of the origin of Chagas disease in humans, approximately 8000–6000 years ago. The high prevalence of *T. cruzi* in pre-Colombian samples of desiccated mummies, some as old as 9000 years, indicated that Chagas disease is probably as old as human presence in the Americas [5].

The rapid extinction (in less than 10 years) of rats endemic to Christmas Island at the beginning of the 20th century was found to be caused by a pathogenic trypanosome, *Trypanosoma lewisi*, carried by fleas present in the recently introduced black rats, where the parasite is not lethal. Molecular analyses of museum-archived endemic rats collected before the black rat introduction revealed that they were trypanosome negative, while those collected after the introduction were positive. The long-isolated endemic rat species were immunologically naive and highly susceptible to *T. lewisi* [6]. With a similar strategy, collection-archived bat specimens would be very useful to investigate the origin and the real extent of *T. cruzi* diversity. All basal species of this clade are parasites of bats [7].

Given that collections can preserve live trypanosomatids, either frozen or through *in vitro* or *in vivo* passages with

a trackable history, they can help to answer questions about old specimens. For example, the etiological agent of dourine in horses, *Trypanosoma equiperdum*, was isolated and described based only on clinical signs, and there are controversies regarding the overlap that occurs between this species with *Trypanosoma evansi* and *T. brucei brucei*. This could be solved using molecular phylogenetic analyses of the preserved samples. However, after more than a century of research, only a few laboratory strains exist, and the history of most of them is unknown [8].

Intriguingly, the stained smears, which are mandatory for studying kinetoplastids based on their morphology, and which are abundant in museum collections, are still little explored. A recent molecular study of trypanosomes of marine fishes from South Africa showed that, despite its morphological diversity on Giemsa-stained smears, only one pleomorphic species was present [9]. Another example is represented by the monoxenic trypanosomatid genus *Rhynchoidomonas*, which was introduced in 1910 to accommodate insect parasites with trypomastigote morphology lacking a pronounced undulating membrane (discussed in [10]). This genus contains seven reported species, of which six were reported in the first half of the 19th century. The last species, *Rhynchoidomonas operophterae*, was described in 1986, also based on morphological characters, and the type slide was deposited at the Natural History Museum in London

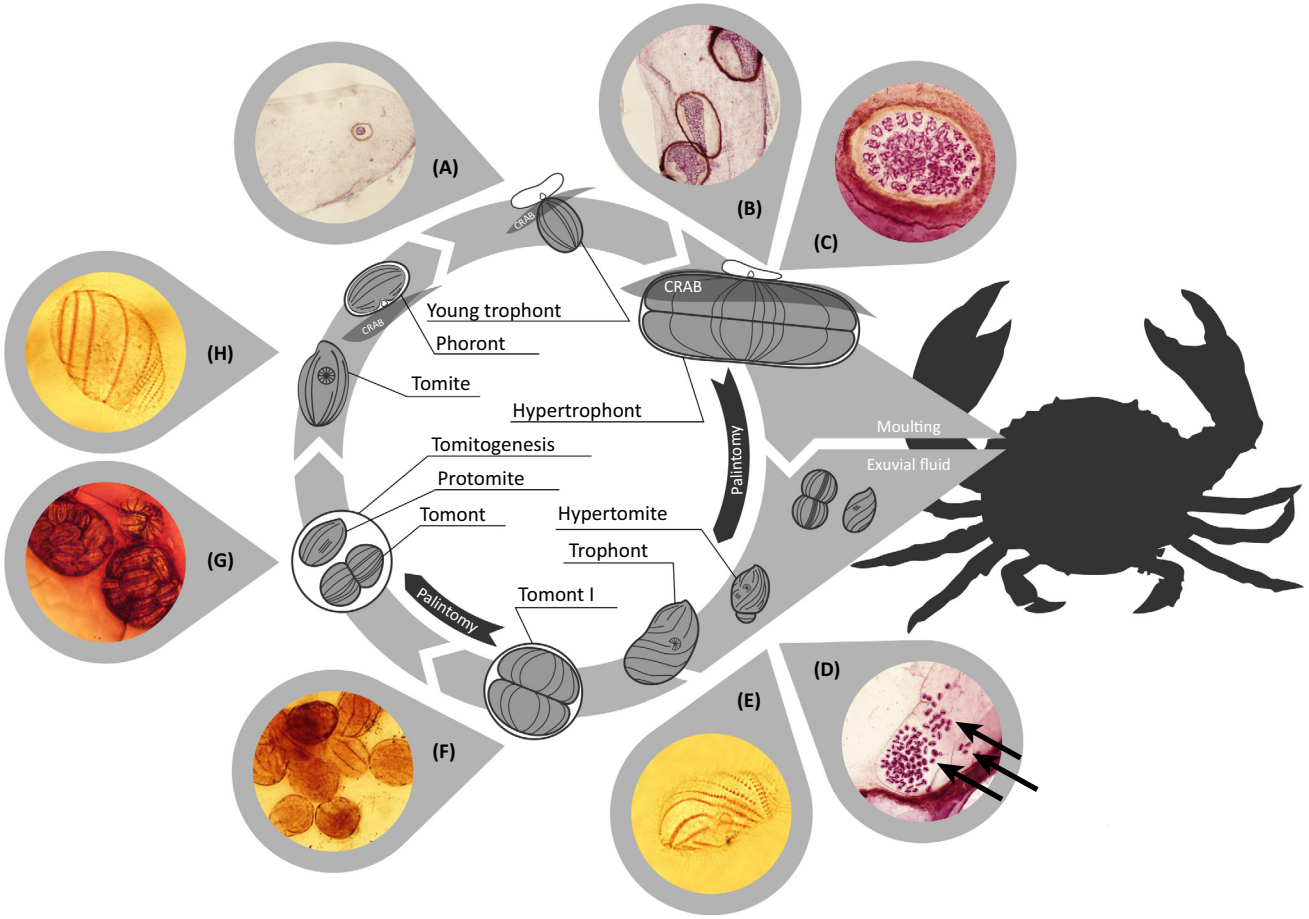

Figure 1. Preserved Type Material in Institutional or Museum Collections. Apostome ciliates are symbiotic protozoa with life cycles including commensalism and parasitism. *Synophrya hypertrophica* Chatton and Lwoff 1926 is an exuviotrophic symbiont infecting crustaceans, which feeds on the exuvial fluid within the exoskeleton after molting. Its monoxenous life cycle was completed by Chatton and Lwoff [12] and has two trophic phases: (i) a histotrophic phase in the gill or under the host exoskeleton; (ii) an exuviotrophic phase at molting. Since 1935 this intriguing parasite has been almost forgotten by the scientific community. Fortunately, hapantotypes preserved in the National Museum of Natural History of Paris, France (150 slides, inventory number MNHN-IR-1970-1) allowed the complete life cycle to be reconstructed and are available for consultation by scientists. (A and B) The invasive swimming tomite encysts (phoront) and invades the gill lamellae of the crab *Portunus*, and grows in size (hypertrophont stage) by feeding on host tissues. The ciliate is isolated from the host tissue by a melanized wall. (C–E) At premolting, the hypertrophont, characterized by a massive reticulate macronucleus, divides by palintomy to produce numerous swimming hypertomites (D, arrows). (F and G) They grow in size by feeding on the exuvial fluid, encyst (tomont stage), and enter division to produce protomites (tomitogenesis). (H) Released from the cyst, protomites differentiate into swimming tomites that propagate the infection. (A–C) hematoxylin staining; (D–H) Chatton and Lwoff silver staining.

[11]. Unfortunately, only one slide was deposited, precluding its use for DNA extraction. Thus, the scientific community still has no means to obtain a molecular signature for this genus.

The value of vouchered deposits is well illustrated for the ciliate *Synophrya hypertrophica* Chatton and Lwoff 1926. The type slide deposits preserved in the National Museum of Natural History of Paris allowed the complete life cycle to be reconstructed (Figure 1).

A link between specimen-derived genetic data to vouchered deposits in publicly-available institution-maintained collections should be the goal of each researcher. The term 'voucher specimens' captures the essential role of the deposits, serving as references for the taxonomy, and further studies. When vouchered deposits are not the first option of researchers to deposit new isolates, the scientific community can face the loss of decades of work of dedicated scientists. To quantify this risk, a survey of the new species description, restricted to the insect monoxenic trypanosomatids from 2000 to 2015, revealed a total of almost 50 new species. Of these, only six were deposited in museum or institutional collections. Furthermore, to anticipate future research needs, the specimen deposits must not only be limited to the hapanotype slides (preparations representing distinct stages in the life cycle of the type specimen), but must also be accompanied by the complementary materials: xenotype of the type host, axenic cultures of the primary isolate and clonal line(s), and total DNA samples from the primary isolate and clonal line(s). Many museums and institutions have, or are developing, infrastructures to support the preservation of such deposits. Through DNA sequencing, digital registries, and other unpredictable advances, existing long-term museum or institutional collections can be interrogated in new ways, revealing more about Earth's natural history. Analysis of recent publications (2015–2016) in two major journals of protistology (*Protist* and *European Journal of Protistology*, $n = 126$ publications) showed that 46.8% ($n = 59$) of articles give no clear reference to the origin or the conservation of the biological materials studied, 14.3% ($n = 18$) used strains from clearly identified collections, and 38.9% ($n = 49$) related to (re)description of new species deposited in collections with inventory numbers. For 73.4% ($n = 36$) of the latter, vouchers were deposited in museum or institutional collections – and for 20.4% ($n = 10$) in at least two different collections. Only for 14.2% ($n = 7$) of the studies, vouchers or type slides were accompanied by a deposit of complementary materials (DNA, cultures, electron microscopy blocks, etc.). Although specimen voucher deposit for new species description is a prerequisite for all major journals, the difference between the two types of collections was never judged. As a result, new species are most often vouchered in laboratory-supported research collections. On the one hand, the scientific community should collaborate more to strengthen the museum or institutional collections; on the other, collections should work harder to publicize their catalogues. The average institution displays only about 1% or less of its store [2].

In conclusion, for the benefit of protistology, vouchered deposits in museum or institutional collections should be pursued by researchers, journal editors, referees, and funding agencies.

Acknowledgments

We would like to thank all the members of our laboratories and participants of the 2nd TryTAX meeting (June 2016, Ostrava, Czech Republic) for helpful and stimulating discussions. We appreciate helpful suggestions on collections policy by Dr Manuela da Silva (VPPLR/FIOCRUZ). V.Y. is supported by the funds of the Moravskoslezský Kraj research initiative (01211/2016/RRC), the Czech Science Foundation (16-18699S), and the project TEWEP LO1208 of the National Feasibility Programme I of the Czech Republic. We thank the Brazilian grant agencies Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (MCT/CNPq), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for funding, as well as Fundação Oswaldo Cruz (FIOCRUZ) for research infrastructure. C.M.D.L. is the recipient of a fellowship from the CNPq and FAPERJ.

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http://dx.doi.org/10.1016/j.pt.2016.08.001

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