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Article

Reconditioning of the Nalepa collection of eriophyoid mites (Acariformes, Eriophyoidea)

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Abstract

Alfred Nalepa (19.XII.1856–11.XII.1929), an Austrian acarologist, described about 460 eriophyoid species. He reported new taxa in short communications usually published in "Anzeiger der Kaiserlichen Akademie der Wissenschaften in Wien" and later prepared detailed descriptions for separate publication. For most Nalepan species the date of the first listing in Anzeiger is the valid date of the taxon name. His archive, library and collection are kept in the Natural History Museum of Vienna, Austria (NHMW). The collection consists of 24 boxes with 1073 vials containing plant material with extracted mites collected during 1887–1929. All boxes are labeled according to the first letters of the host-plant names and are sorted alphabetically; the vials are numbered and labeled. A jotter, presumably representing the hand-written catalog of the vial collection, and work diaries, containing indications of numbers of the vials, were found in the Nalepa archives. Nalepa used picric acid, hydrochloric acid, ethanol, formalin and creosote for preservation of mites. In all vials the preservative totally evaporated so that only dry sediment remains at the bottom of the vials. A solution containing ethanol, ether and acetic acid was found to be appropriate for dissolving the sediment. A simple, fast protocol for recovering mites from vials and making good slides was developed. It includes four steps: 1) opening the vial; 2) dissolving the sediment; 3) treating mites in lactic acid; 4) slide mounting. All digital data obtained from the Nalepa archives (database of the vials, copies of the jotter, reprints and drawings of mites) will be available for scientists at the web site of NHMW http://www.nhm-wien.ac.at/en/nalepa.

Key words: new protocol, recovery, taxonomical revision, phytoparasitic arthropods

Introduction

Doctor August Nemesius Alfred Nalepa (19.XII.1856–11.XII.1929) was an Austrian acarologist famous for his pioneer studies of tiny phytoparasitic mites of the superfamily Eriophyoidea. Bibliographic data on this scientist can be found in two obituaries (Masee 1930, Rechinger 1930) and in a very sincere paper by Shevtshenko (1967) dedicated to the 110th anniversary of the birth of A. Nalepa. Systematization of the knowledge on eriophyoids obtained by European acarologists in 19th and early 20th centuries and faunistic studies are essential contributions by A. Nalepa to acarology, which contributed greatly to the development of eriophyoidology in the middle of 20th century. According to Keifer (1975) and Newkirk (1984) A. Nalepa published 113 papers and described about 330 new species. Elevation of many of his trinomials and tetranomials has resulted

in about 462 named mites. Fundamental observations on anatomy, ecology and taxonomy of eriophyoids by A. Nalepa (for details, see Shevtshenko 1967 p. 470 and Keifer 1975 pp. 329–332) influenced many scientists and inspired them to study eriophyoids in different countries and continents after his death. At present it is hard to find a paper on eriphyoid mites which does not cite at least one of Nalepa's papers.

Along with his papers, the private archives and scientific collections are the most valuable heritage left by Dr. A. Nalepa to his followers. This important collection was shrouded in mystery for decades and was thought to be lost (Shevtshenko 1967) until it was discovered being kept in three small cupboards in the Natural History Museum in Vienna, Austria, under curation by Dr. J. Gruber (Amrine and Manson 1996, p. 386). Since then Dr. J. Gruber has retired and in 2007 a new curator, C. Hörweg MSc, replaced him. The collection includes reprints of Nalepa papers, his personal library (mainly books and papers on eriophyoids by Nalepa's contemporaries) and numerous glass vials. Only dried sediment is present on the bottom of the vials, whereas the preservative medium had totally evaporated (Amrine *et al*. 2003). All of these conditions greatly challenge the identification and search for eriophyoid mites in the Nalepa collection. Several researchers attempted to recover mites from the vials and mount them to get neotypes (de Lillo *et al*. 2010, p. 288; Xue *et al*. 2015, p. 77; Dr. D. Knihinicki and Dr. C. Craemer personal communications, 2014). Only once it was successful and resulted in slides of *Phytoptochetus tristichus* Nalepa 1917 which were good enough to assign a neotype and draw the mite (Amrine *et al.* 2003, pp. 2, 50, fig. 50). However the protocol developed for recovering and mounting *P. tristichus* was quite complicated and clearing took a period of two months (de Lillo *et al*. 2010, p. 288) implying that development of an easier and shorter protocol is very important.

In 2012 a group of eriophyidologists attending the VIIth EURAAC symposium in Vienna (Drs. C. Flechtmann, R. Petanović, D. Navia, B. Vidović, C. Craemer and P. Chetverikov) visited the Nalepa collection and concluded that it should be carefully reconditioned and an optimal procedure for recovering mites and making neotypes should be developed. In 2014 a two year project "Reconditioning of the Nalepa collection at NHMW" was initiated. This project was primarily aimed to recondition the Nalepa collection in order to make it available for a broad range of scientists. The main goals of this study were: 1) to evaluate, describe and improve the current condition of the collection; 2) to develop the best technique for recovering mites from vials and making slides; 3) to make a digital database of vials from the Nalepa collection and 4) to find the best way for preserving the collection for the future. In this paper we present the most significant results obtained during the project which will be important for eriophyoid studies.

Material and Methods

Reconditioning of the collection and databases. All the boxes containing vials were labeled according to the first letters of the host-plant names (A,B,C etc.) and sorted alphabetically. Inside every box the vials containing material from one plant genus were put together. Those vials containing damaged plant organs (but not sediment) were additionally marked with a red dot. The deformed old cork stoppers not properly fitting to the vials were removed, stored in a zip-plastic bag; they were replaced by new ones (VWR® Cat. No. 217-1017 & 217-1016) and new carton labels with numbers were glued (with UHU® hart, Art. Nr. 45510). All data from labels which was possible to recognize were entered into an Excel file and a database of all the vials was created. Using reprints from the Nalepa archive and data from Rechinger (1930), Shevtshenko (1967) and Newkirk (1984) we compiled the full list of papers by A. Nalepa following the system of Nalepa's papers proposed by Newkirk (1984). Digital copies (pdf files) of most of these papers were obtained from our

personal collections and completed with several rare papers which were found in the Nalepa archive and scanned at NHMW. Additionally all the images from Nalepa's papers and some of his original drawings of mites were scanned with high resolution (300 dpi, half-tone) and saved separately as .pdf files.

Testing methods for recovering mites from sediment and making slides. For recovering mites from the vials we searched for mites under a stereomicroscope Leica MZ6 in the solutions obtained by dissolving the sediment from the vials in various cold and hot media (distilled water, 70% ethanol, 5% acetic acid, diethyl ether $(C_2H_5)_2O$ and mixtures of all these chemicals in different combinations). For making permanent slides we applied modified Berlese medium (Amrine and Manson 1996) and tested pretreatment in 20% KOH or technical DL-lactic acid. For heating the solutions with sediment and slide mounts we used a Heraeus EW-BAL Thermostat Oven; different temperature (from 70°C to 115°C) and time (from 30 minutes to 72 hours) were tested. The final and best protocol for recovering mites is described below under the section "Protocol developed in this study". Photographs were obtained using a Nikon Coolpix R310, and microphotographs of the recovered mites and crystals inside vials were obtained using a stereomicroscope Nikon SMZ25 equipped with a microscope camera Nikon DS-Ri2. Quality of the slides was checked using a conventional light microscope Leitz Diaplan.

Results

General characteristics of the collection (Fig. 1, Tab. 1). All of Nalepa's materials are located in three cupboards (#90, #91, & #92). All the papers from the Nalepa's library written by his precursors and contemporaries (Thomas, Trotter, Schlechtendal etc.) are in the upper part of cupboard #90. The boxes with vials (mainly material from Austria), glass bottles and envelops (with material from Samoa, Java, New Zealand, Australia and Barbados) are in the cupboard #91; a small plastic box containing the type specimen of *Phytoptochetus tristichus* is also in this cupboard. In total, there are 24 boxes (23 square, wooden boxes about 20x20x20 cm and one white carton box of larger size) containing 1073 vials collected from 1887 (the oldest record) till 1929 (the last record). All the boxes are labeled according to the first letters of the host-plant names (A,B,C etc.) and sorted alphabetically. Inside every box, the vials containing material from one plant genus are kept together (Table 1). In the cupboard #92 the original pencil drawings, reprints of Nalepa's papers and his correspondence and diaries are kept.

Current condition of the vials. The vials are about 8–10 cm long and 12–14 mm wide. The corks are hard to remove from the vials because Nalepa dipped their bottom portion in paraffin prior to inserting into the vial (Nalepa 1906, p. 58). Paper circles with numbers written in ink or pencil are glued to the top of most corks; sometimes an index note is added (e.g. "64a" or "323bis"). Labels on the outer walls of each vial contain at maximum the following information: name of host plant, type of deformation or any other data on relation to the host, mite species, number of the vial, locality and a date (Fig. 2). Mite species and locality are missing in many labels. The date is provided usually as a year; it is usually the date of collecting; however in several cases it is distinctly the date of the publication for the description of the mite species in the vial. Terminology of the plant deformation is given in German, it can be retrieved from Nalepa (1887, p. 149) or from his later publications. There is a problem with host-plant names used by Nalepa because of the old plant terminology. However careful searching of botanical literature and botanical databases, such as USDA Plants, GRIN, Tropicos, BONAP, Flora of China, Kew Gardens, the Plant List, and others, will help find the correct contemporary name. At least three times in his life Nalepa entirely revised his collection to prepare large reviews and catalogs. It may be for this reason that many labels show two numbers;

one of them preceeded by the letter “K”, suggesting possible reference to a lost catalog (“Katalog” in German) or his 1929 published Katalog. Several vials contain an additional small label inside indicating the reference for the description of the mite species and/or detailed collecting data.

FIGURE1. Photographs of the cupboards (on the right) and boxes with vials inside cupboard #91 (on the left).

FIGURE 2. Microphotographs showing a vial, cork, label and the reconstructed text from the label (from left to right).

The content of the vials. Nalepa (1906, p. 56) reported that he added three different media to the vials: #1 a mixture of picric acid, distilled water and concentrated hydrochloric acid, 1:100:2 (“Pikrinsalzsäure”); #2 a mixture of 94% ethanol and concentrated hydrochloric acid, 100:2 (“Säurealkohol”) and #3 80% ethanol. The media #1 and #2 were used both for extracting mites (by shaking plant material with medium in a tube) and preservation; prior to preservation in the vial, mites in media #1 and #2 were diluted in 1:4–5 with water and heated to 50–60°C. Medium

TABLE 1. Characteristics of the boxes with vials from Nalepa's collection. *Note*: plant genera are given exactly the same as indicated in the labels, using the spelling style of the time (e.g. *Evonymus, Gossipium*).

Box	Number of vials in a box	Genera of plants mentioned on the vial labels (number of the vials from exact plant genus is mentioned in brackets)
A1	59	*Abies* (3), *Acacia* (1), *Acer* (55)
A2	45	*Acer* (45)
A3	37	*Aesculus* (4), *Ajuga* (2), *Alnus* (31)
A4	42	*Alyssum* (2), *Amelanchier* (2), *Anchusa* (2), *Andromeda* (1), *Aposeris* (3), *Arabis* (3), *Arctostaphylos* (2), *Artemisia* (16), *Asperula* (5), *Atragene* (1), *Atriplex* (2), *Avena* (1), *Azalea* (2)
B	45	*Bartsia* (1), *Bellidiastrum* (1), *Berberis* (2), *Bertoroa* (1), *Betonica* (1), *Betula* (21), *Brachypodium* (1), *Bromus* (2), *Bucida* (2), *Buxus* (13)
C1	54	*Camellina* (2), *Campanula* (8), *Carlina* (1), *Carpinus* (11), *Carum* (2), *Cedrus* (2), *Centaurea* (7), *Cerastium* (1), *Chondrilla* (4), *Cinnamomum* (1), *Cirsium* (1), *Cistus* (1), *Clematis* (5), *Convolvus* (3), *Cornus* (2), *Coronilla* (2), *Cydonia* (1)
C2	48	*Colutea* (2), *Corylus* (22), *Cotoneaster* (6), *Crataegus* (14), *Crepis* (1), *Cytisus* (3)
DE	26	*Dactylis* (1), *Doryconium* (1), *Echinospermum* (2), *Echium* (2), *Empetrum* (1), *Erigeron* (1), *Erodium* (3), *Ervum* (1), *Erysimum* (1), *Eugenia* (2), *Eupatorium* (2), *Euphorbia* (1), *Euphrasia* (2), *Evonymus* (6)
F	39	*Fraxinus* (13), *Fragaria* (4), *Fagus* (22)
G	27	*Galium* (27)
GHIJ	47	*Genista* (1), *Gentiana* (3), *Geranium* (5), *Geum* (5), *Gossipium* (2), *Helianthemum* (3), *Hibiscus* (4), *Hieracium* (4), *Hippophae* (1), *Hutschinsia* (1), *Hypocharis* (1), *Ipomoea* (1), *Juglans* (7), *Juniperus* (6), *Jurinea* (2), unknown (1)
L	31	*Lactuca* (1), *Larix* (3), *Laurus* (2), *Lepidium* (2), *Linosyris* (1), *Lonicera* (7), *Lotus* (4), *Lycium* (2), *Lycopsis* (1), *Lysimachia* (8)
MNO	26	*Malva* (2), *Mangifera* (2), *Medicago* (2), *Mentha* (2), *Moehringia* (2), *Molinia* (1), *Ononis* (2), *Origanum* (8), *Orlaya* (1), *Ornithopus* (2), *Oxalis* (1), unknown (1)
P1	54	*Paederota* (1), *Passerina* (1), *Pedicularis* (1), *Peucedanum* (1), *Phlomis* (3), *Pimpinella* (3), *Pinus* (6), *Pistacia* (3), *Plantago* (1), *Potentilla* (3), *Pyrus* (31)
P2	56	*Polygala* (2), *Populus* (19), *Poterium* (3), *Prunus* (30), *Pteris* (2)
QR	47	*Quercus* (18), *Robinia* (3), *Ranunculus* (4), *Rhamnus* (3), *Rhodiola* (3), *Rhododendron* (4), *Rosa* (2), *Rubia* (2), *Rubus* (2), *Ribes* (6)
S1	61	*Salicornia* (1), *Salix* (60)
S2	44	*Sambucus* (8), *Sarothamnus* (2), *Saxifraga* (3), *Scabiosa* (2), *Serratula* (1), *Seseli* (2), *Sisymbrium* (2), *Solanum* (3), *Sonchus* (1), *Sorbus* (20)
S3	33	*Salvia* (7), *Sedum* (8), *Spiraea* (1), *Spiraeanthemum* (1), *Staphylea* (1), *Stellaria* (3), *Suaeda* (2), *Symphyandra* (1), *Syringa* (9)
T	59	*Tilia* (59)
TU	48	*Tamarix* (1), *Tanacetum* (3), *Taraxacum* (3), *Taxus* (1), *Teucrium* (1), *Thalictrum* (1), *Thesium* (2), *Thymus* (4), *Torilis* (2), *Trifolium* (1), *Trinia* (1), *Triticum* (1), *Ulmus* (27)
V	37	*Veronica* (6), *Viburnum* (11), *Vicia* (2), *Viola* (3), *Vitex* (1), *Vitis* (14)
Java-I	48	*Acacia* (2), *Acalypha* (1), *Acronychia* (1), *Alangium* (1), *Allophyllus* (1), *Aporosa* (1), *Bauhinia* (2), *Beilschmiedia* (1), *Buettneria* (1), *Callicarpa* (1), *Canarium* (1), *Cinnamomum* (1), *Clerodendron* (2), *Conocephalus* (1), *Cordia* (1), *Crotalaria* (2), *Cryptocarya* (2), *Dianthera* (1), *Ehretia* (1), *Elaeocarpus* (2), *Evodia* (2), *Ficus* (7), *Flacourtia* (1), *Fluggea* (1), *Glochidium* (5), *Gonania* (1), *Haasia* (=*Dehaasia,* 1), *Hibiscus* (4)
Java-II/1*	12	*Indigofera* (3), *Ipomoea* (2), *Laportea* (2), *Lepistemon* (1), *Litsea* (3), unknown (1)
Java-II/2	13	*Macaranga* (1), *Macropanax* (1), *Mallotus* (2), *Melastoma* (1), *Melochia* (1), *Merremia* (1), *Mikania* (1), *Morinda* (1), *Nephrolepis* (3), *Oldenlandia* (1)
Java-II/3	17	*Paederia* (1), *Pavetta* (1), *Peristrophe* (1), *Pluchea* (1), *Pometia* (1), *Premna* (2), *Pterospermum* (1), *Quisqualis* (1), *Rubus* (1), *Ruellia* (1), *Sandoricum* (2), *Sesbania* (1), *Solanum* (1), *Streblus* (1), *Strobilanthes* (1)
Java-II/4	18	*Tetracera* (1), *Toddalia* (1), *Triumphaeta* (3), *Unona* (1), *Vangueria* (1), *Villebrunnea* (3), *Viburnum* (1), *Vitex* (2), *Weinmannia* (4), *Wendlandia* (1)

* the box Java-II has four compartments: II/1, II/2, II/3 and II/4.

#3 was used for preservation of galls and other damaged tissues caused by mites. Nalepa also used to add a small amount of creosote in the vials to avoid microbial contamination. Besides these chemicals, "formalin" is mentioned in several labels suggesting that it was also used as a preservative. However in most labels indication of the medium is absent so that it is not possible to know which medium was actually applied.

Only dry sediment is present now in most of the vials. It is very dense, usually dry and can be crushed into small pieces only by using a strong needle. In several vials the sediment is viscous resembling resin or oil. This may be because of possible polymerization of formaldehyde. The color of the sediment varies: usually it is dark or light brown, sometimes gray, dark gray or almost black. Green, yellow and brown crystals are present in many vials (Fig. 3), which are most probably crystalized salts of picric acid. Yellow crystals are especially numerous in the series of vials containing material from *Tilia* spp. (about 60 vials). About 12% of all the vials (127 vials) contain small dry pieces of plants (fragments of galled leaves, buds and inflorescence). These vials are marked with a red circle on a cork. Although the mummies of the mites can be easily found in the dry plant material, it is better to process such material similar to the sediment (see below) to make the mites more translucent because, similar to the mites from sediment, a dark sedimental material is also present inside them.

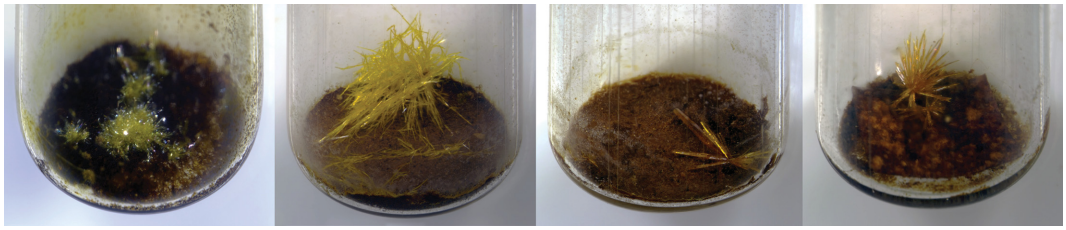

FIGURE 3. Microphotographs showing the sediment with crystals inside the vials.

Work diaries and hand-written catalog (Fig. 4, Fig. 5). Ten small notebooks are present in the archives (Fig. 4); these are work diaries of Nalepa. Four of them are not numbered, but on the hardcovers of all the others, numbers are indicated. The dates of the beginning of most diaries are given in the first page (#1—1895; #2, #3—unknown; #4—14.II.1914; #5—5.X.1916; #6—22.I.1916; #7—25.III.1917; #8—unknown; #9—9.IV.1920; #10—15.IV.1922). Measurements, draft images of prodorsal shields and short descriptions of eriophyoid mite species in pencil can be found in these diaries; for many species the numbers of corresponding vials are indicated.

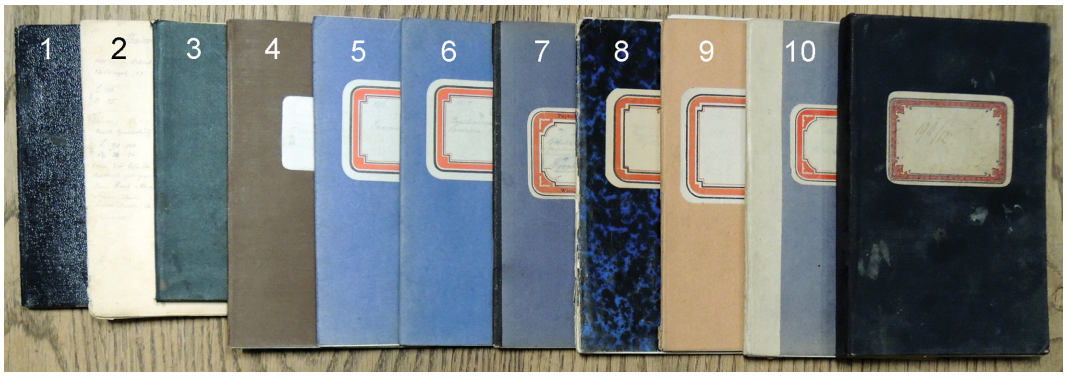

FIGURE 4. Ten work diaries (numbered) and a putative catalog (the black jotter).

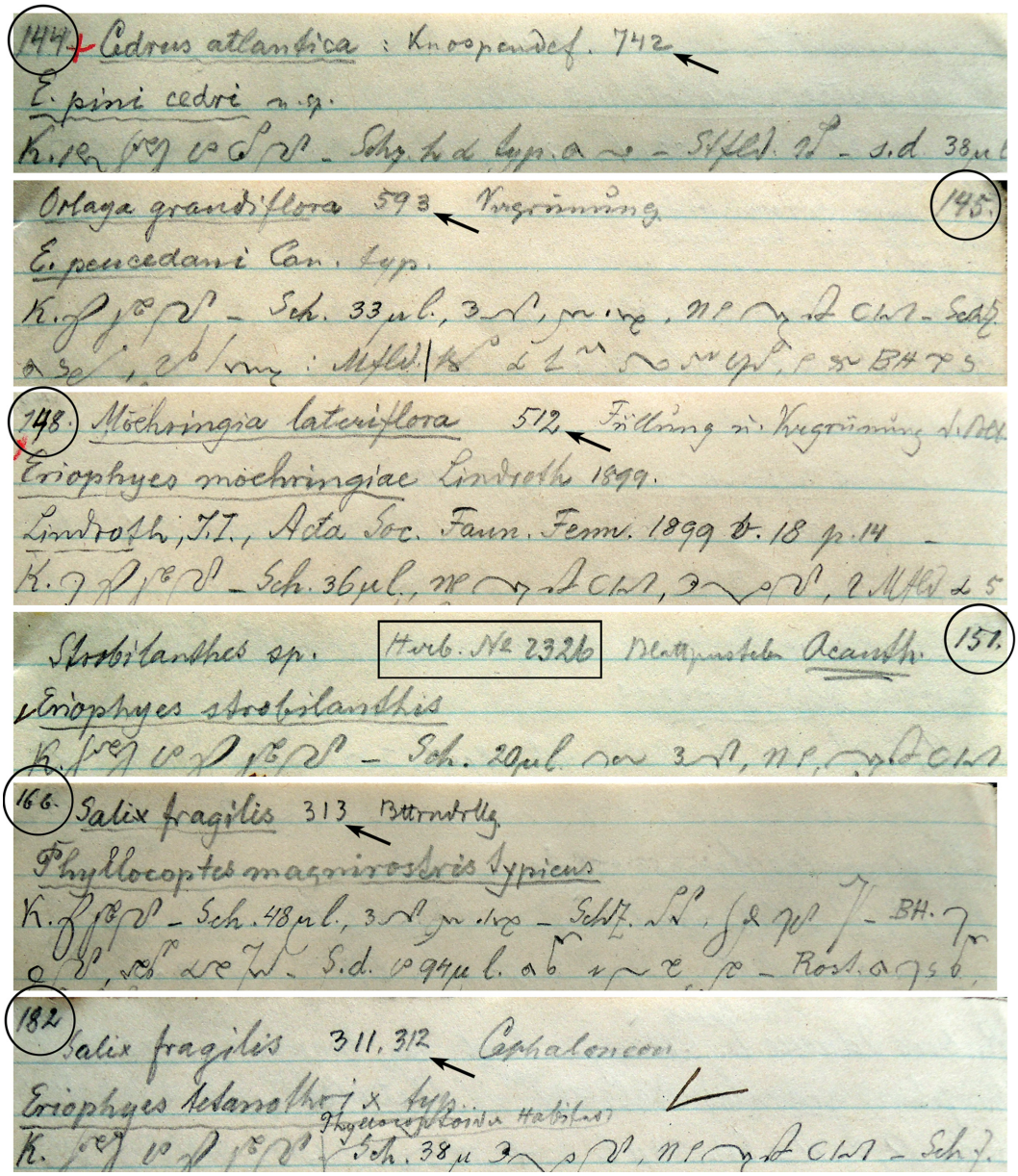

FIGURE 5. A photograph showing six examples of the putative hand-written catalog with page (black circle) and vial numbers (arrow) indicated. The reference to a herbarium sample is indicated by a black rectangle.

Along with the work diaries another important document was found in the Nalepa archives (Fig. 5). It is a small jotter of 220 pages (each page is enumerated by hand) with black hardcover. Although the date "1911/12" is indicated in the hardcover, this document contains information about material from Java suggesting much later date of use. We assume that this jotter is possibly the very hand-written catalog mentioned by previous authors (Amrine and Manson 1996; Amrine *et al*. 2003; de Lillo *et al*. 2010) and which was thought to be lost. The texts in the jotter are written in pencil in an old-fashioned mode using "cursive writing" (stenography) with a lot of abbreviations, making it hard to understand. However, comparison with the two catalogs published by Nalepa (1911, 1929)

revealed that the jotter might have been a draft for these publications as most descriptions from the jotter precisely fit to the texts from the two published catalogs. Many species descriptions (but far from all of them) include indication of the numbers of vials with corresponding material (Fig. 5, arrows). On page 157 a reference to a herbarium specimen of *Strobilanthes* sp. is given in the description of *Eriophyes strobilanthis* (Fig. 5, rectangle); the herbarium specimen and the vials with this mite species have not been found in the collection.

The jotter consists of four parts: pages 1–58; 60–137; 138–196 and the last unnumbered 24 pages. In the first 58 pages the descriptions of new mite species from Java collected in 1914 are given, the host index is provided on pages 55–58. Pages 60–134 contain descriptions and records of European species of eriophyoids, host index for these species is given on pages 135–137. On pages 138–196 descriptions of new species from Java collected in 1921 as well as data on European species are given in a jumble; another host index for mites from Java is given on pages 179–180. The last part of the jotter contains some unfinished tables and notes on the mites from *Acer* spp. with pencil drawings showing different forms of the modified trichomes of the erinea caused by eriophyoids on *Acer pseudoplatanus* and *A. campestre*; several pages are blank. Careful, future study of this jotter and the work diaries will be necessary to better understand the content of each vial from the collection.

Database of the vials and other digital data obtained. The following digital data were prepared: 1) pdf copies of 108 Nalepa papers and 2) pdf copies of the jotter and ten work diaries; 3) a list of the eriophyoid mite species and genera described by A. Nalepa; 4) high resolution copies of the figures from all Nalepa papers and his original pencil drawings of the mites; 5) pdf copies of the papers about A. Nalepa and his work; 6) photographs of the collection and 7) database of the vials. The database organization is shown in Table 2. All the digital data are available for scientists through the Internet at the NHMW site http://www.nhm-wien.ac.at/en/nalepa.

TABLE 2 A portion of the Excel file showing the organization of the database

Box #	Vial #	Plant genus	Plant species	Damage	Mite(s) Species	Coll. date	Locality	Content
C2	180	*Crataegus*	*oxyacantha*	Knospendef.	*P. calycobius*	1891?	Gmunden	dark brown sediment
DE	190	*Evonymus*	*europeus*	Blattrandroll.	*Cecidophyes convolvens*	1889?	?	dark sediment
GHIJ	130a	*Galium*	*aparine*	?	?	VI.1926	?	brown sediment
GHIJ	639	*Geranium*	*palustre*	Erineum def. Blt	?	1908	?	leaf with erineum

Technique for recovering mites from vials and making slides. Analysis of the literature revealed that previous authors scrabbled dry sediment on the bottom of a vial, removed it and looked for the mites under a stereomicroscope. According to our experiments this is the reason why most of the previous attempts to recover mites were unsuccessful because 1) the mites are usually tightly embedded into the sediment and thus are hard to be visualized and 2) scrabbling dry sediment often results in crumbling the mites (not only breaking legs and setae but also totally destroying the exoskeleton). The protocol developed in this study implies that the sediment should be liquefied and/or dissolved and then the mites are collected. Following this protocol about 80% of the resulting slide mounts are of good quality and appropriate for studying morphology of mites, and is quite similar to mounting live mites, although more time consuming. The protocol includes four steps: 1) opening a vial; 2) dissolving the sediment; 3) treating mites in lactic acid; 4) slide mounting. Description and remarks on these stages are as follows:

1. Opening the vial (Fig. 6). The old cork usually breaks when being removed so that the lower portion of the cork remains inside the vial (Fig. 6A). It can be removed by sharp, evenly tapered forceps. Thrust the forceps into the broken cork at an angle (as shown in Fig. 4B) and twist as with a corkscrew; the cork will be gradually cut out, piece by piece. Keep the vial horizontal to avoid cork pieces falling into the vial. Remove remnants of paraffin (Fig. 6A, arrow) from the inner surface of the vial with a scalpel. Insert a new cork and glue a label with the cork number from the old cork. If the number was written in pencil and is indistinct it can be revealed by adding drops of ethanol.

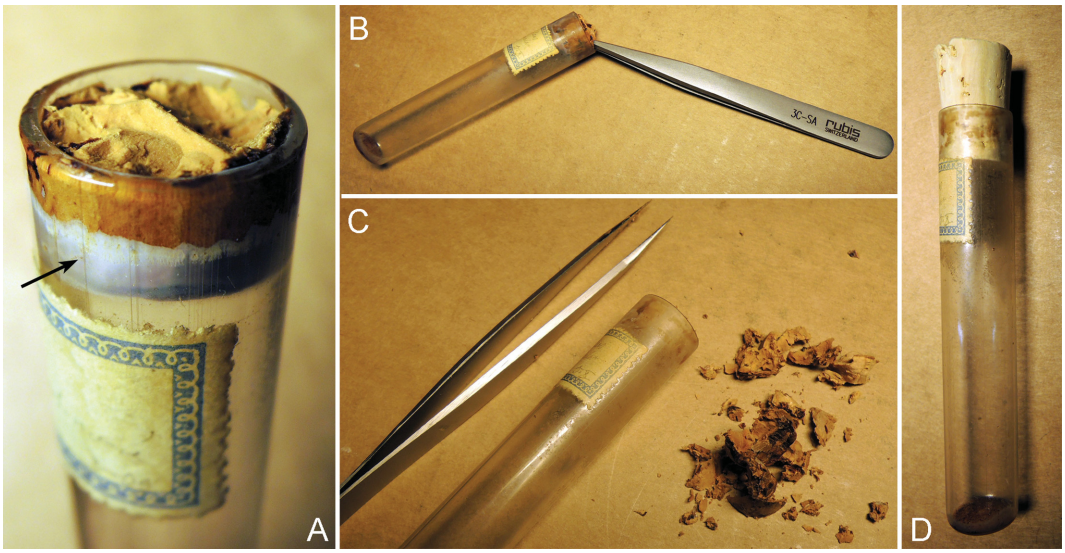

FIGURE 6. Opening the vial. A—vial with broken cork; B, C—removing lower portion of the old cork by screwing the inserted forceps; D—the vial with new cork and dry sediment on the bottom. *Note*: black arrow indicates paraffin.

2. Dissolving the sediment (Fig. 7). Add 5 ml of 70% ethanol, 3 drops of 5% acetic acid and 10 drops of pure diethyl ether into the vial. Heat for about 4 hours at +75°C. Shake the vial to check if the sediment has been dissolved. If not, heat until it is dissolved. In all of the studied vials the sediment dissolved or turned into soft flakes within 24 hours.

Remarks. Ethanol boils at +78.29 °C (Haynes, 2014), thus avoid heating the vials at temperatures above 75°C. When formalin evaporates, it forms different resinous compounds (polymers with structure $[-OCH_2-]_n$), which can be hydrolyzed under slightly acid conditions (pH<7) (Utterback et al. 1984). It should be mentioned though, that these polymers can be also hydrolized by alkaline solutions (pH>7) but this can lead to undesirable oxidation processes (Smith & March 2013), therefore acid conditions are preferable. Phenolic components of creosote (like guaiacol or creosol) are soluble in ethanol and ether; salts of picric acids are soluble in ethanol (Rappoport 2003). Therefore combining ethanol, acetic acid and ether in one mixture for dissolving sediment is warranted. Prior to the next step the mites can be additionally treated with KOH. Mites from oily plants (*e.g.* from conifers) sometimes better clear after KOH treatment, however in most cases treating with KOH can be omitted.

3. Treating mites in lactic acid. Pipette about 0.3–0.5 ml of the solution from the vial to a small glass stender dish with a hemispherical cavity. Observe the sediment under a stereomicroscope, the mites should be on the bottom (Fig. 8). If the mites are dark orange or brown and the solution is bright yellowish, additional heating can be applied. In this case add about 3–5 ml of 70% ethanol into the

dish, cover with a square glass to prevent evaporation and heat for about 10–12 hours at +75°C. Afterwards, fish the mites out with a needle and put them (one mite per one glass slide) in a small drop of lactic acid (about 1 mm in diameter). Encircle the spot with mites by a marker on the backside of the glass slide and heat for several hours at +95°C.

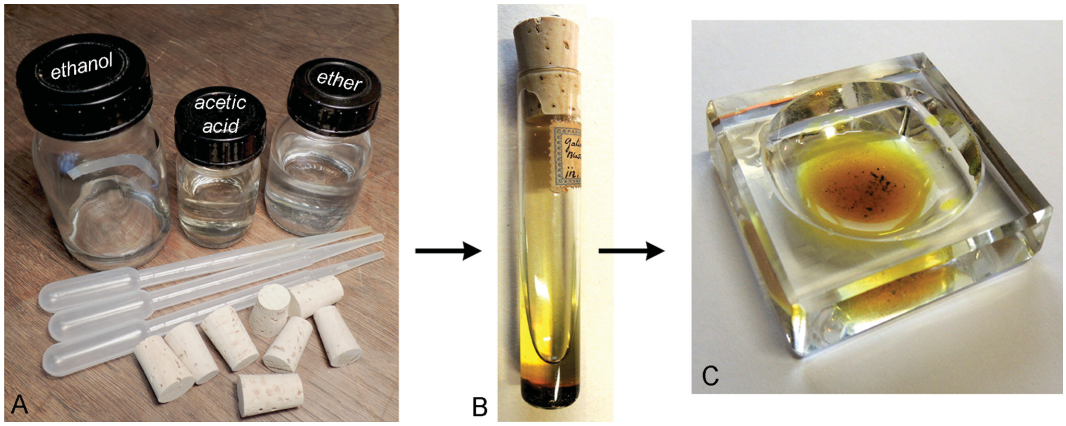

FIGURE 7. Dissolving the sediment. A—reagents for dissolving sediment; B—a vial with dissolving solution prior to heating (undissolved sediment on the bottom of the vial); C—solution with dissolved sediment in a stender dish after heating.

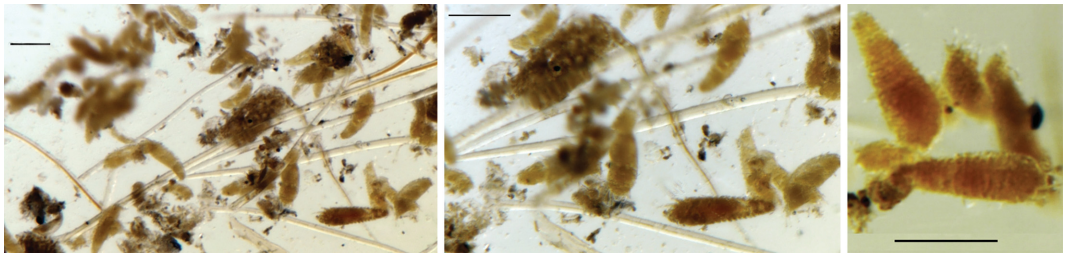

FIGURE 8. Mites in the solution with dissolved sediment. Scale bar = 100 μm.

Remarks. The boiling point of technical DL-lactic acid is 122°C (NCBI PubChem, 2015). Mites from several vials could be cleared only by applying temperatures of about 100–110°C and for a long time (up to 18 hours or even overnight), however most mites were appropriately cleared after treating in lactic acid for about 4–6 hours at +95°C. It is important that most of the lactic acid evaporates so that only a thin plane, "mirror" spot of lactic acid with mites remains on the slide. At this stage the mites slightly adhere to the glass and will not notably change their position when being slide mounted (during step #4). From time to time, check the mites to avoid total evaporation of the lactic acid, otherwise the mites might be damaged. If lactic acid is close to total evaporation, a new small drop can be added above the mites.

4. Slide mounting. Put a small drop of modified Berlese medium (Amrine and Manson 1996) onto the mite, put a small drop on the coverslip (to prevent air bubbles) and place the coverslip onto the mite and move the coverslip to orient the mite properly. Heating at +85°C for 12 hours or overnight is recommended. Seal the slide and label it.

Discussion

Although most previous attempts to recover mites from the vials were unsuccessful suggesting unsuitability for being used in taxonomical studies, this study shows that Nalepa's collection is still of great value as mites can be recovered from most vials using quite simple but tedious methods. The protocol developed in this study allows obtaining good slides after 3–5 days, which is considerably faster than was thought before (Amrine and Manson 1996; de Lillo *et al*. 2010). Along with the collection of the vials, the personal library of Nalepa is of great importance. It includes a lot of rare publications containing first designations and short descriptions of many common European eriophyoid mite species which were mentioned by Nalepa in his papers when he compared new species with old ones. Additionally, A. Nalepa often wrote abbreviated references in the vial labels indicating the paper with the description of the species from the vial (e.g. "Th 89" = Thomas, 1889 or "Rubs. 11 " = Rubsaamen 1911). This information is helpful when trying to understanding the content of a vial.

Professor Nalepa usually announced his new taxa in short communications usually published in "Anzeiger der Kaiserlichen Akademie der Wissenschaften in Wien". Only 1–5 years later he prepared detailed descriptions of these taxa (with figures of mites and sometimes drawings of the damaged plants) in a separate, longer publication. As a rule, in the short communications a new mite name, host plant species and the damage caused by the new mite were indicated; for inquiline and vagrant mites only host plant names were given. Xue *et al*. (2015, p. 72) considered the date of the later publication to be the correct date of the species name and mentioned that they followed Newkirk (1984) in this question. However, two cases should not be mixed: #1, if a new species name is accompanied with the name of the host plant and there is an indication of the damage caused by the mite and #2, if only a new mite name and host name are provided and no plant injury is indicated. Case #1 corresponds to the article 12.2.8 of the International Code of Zoological Nomenclature (ICZN 1999) because an "indication" is present and includes "the description of the work of an organism". Therefore the new mite name from case #1 is the valid name. However, case #2 does not follow the Code, thus the new mite name is not valid.

Newkirk (1984) reported that "…Nalepa published descriptions of 331 species, 42 varieties and 28 subspecies". According to our preliminary estimation Nalepa described about 460 new species (≈9% of the currently described number of eriophyoid species) from 42 genera from 3 families: Phytoptidae (3 genera/10 species), Diptilomiopidae (4 genera/8 species), Eriophyidae (35 genera/ 442 species). The precise estimation is difficult for several reasons: 1) various acarologists gave specific status to a number of Nalepa's species and varieties; 2) different forms of one species (*e.g.* deutogyne and protogyne females) sometimes were considered by Nalepa as different species; 3) in Nalepa's time the standards for description of new eriophyoid species were poor and many authors described mites based on a short vague textual description and images of the damages which the mite caused on plants; as a result the same species was sometimes described by different authors (including Nalepa) under different names. Thus careful revisions of old European species and comparison with type material are needed for accurate determination of the exact number of species described by Nalepa. Two groups of species from Nalepa's collection are of especial importance: 1) the type species for genera and 2) economically important mite species. Although most of the species described by A. Nalepa are from Europe, a very important part of the collection represents material from non-European regions, mainly from Java, but also from Fiji, and other locations. Due to intensive work by Chinese, Thai and Indian acarologists in the last decades, eriophyidology has been very quickly developing in Asia in the XXIst century (de Lillo *et al*. 2010; de Lillo & Skoracka 2010). Therefore the material from Java may be of especial interest for taxonomic revisions by Asian specialists. It should be mentioned though, that among the vials from Java, three important vials

containing type material of *Cecidodectes euzonus* Nalepa 1917 (type species of genus *Cecidotectes* Nalepa 1917), *Diptilomiopus javanicus* Nalepa 1917 (type species for genus *Diptilomiopus* Nalepa 1916) and *Eriophyes strobilanthis* Nalepa 1921, were not found; this material is probably lost. Collection of these species in type localities and designations of the neotypes will be necessary.

The scientists who are interested in recovering mites from Nalepa's vials or depositing type material at NHMW are welcome to contact the curator of the collection via e-mail (*christoph.hoerweg@nhm-wien.ac.at*). It is preferable if the recovering process is performed at NHMW; in this case the scientists might consider a 4–5 day working visit to NHMW. All the materials after recovery should be saved and all the slides should be appropriately labeled, catalogued and kept in the collection. The most promising approach to work with the collection is revising groups of vials from the same host plant. In this case a limited number of possible species will be recovered. They can be easily identified based on the original descriptions especially those species which had been described before 1910. The reason for this is, that after 1910, Nalepa did not provide figures of the mites in his descriptions. However, in general, his later descriptions seem to be more detailed which slightly compensates for the absence of figures. Brief analysis of the new database for the vials suggests that usually, the higher the number indicated on the vial cork the later it was collected. This fact simplifies searching the relevant papers containing descriptions of the possible species.

In the beginning of this study it was hoped that the current condition of the collection might be improved by adding an appropriate solution to all the vials. After developing the protocol for recovering mites, we concluded that it is better to keep the vials as they are. Besides Nalepa's collection, there are several more important old collections of eriophyoids (e.g. slides and envelopes with plant material of H. H. Keifer in USA, "Cecidotheca Italica" in Italy, "Cecidotheca Rossica" in Russia and others). Accurate revisions and making digital profiles of these collections is one of the important goals for the future. Every year dozens of new species of eriophyoids are described worldwide (de Lillo & Skoracka 2010), not all of the descriptions are of appropriate quality. To avoid future chaos in eriophyoid taxonomy it is important to decrease the speed of creating inadequately described new taxa, to intensify faunistic surveys for revising long forgotten information by previous authors (e.g. a study by Hellrigl (2003) for old Trotter species), in order to update the nomenclature and to involve older material in taxonomical studies.

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