



International **Andrea Cesalpino** Systematics Symposium

# Book of Abstracts

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# Invited Lectures

## **Andrea Cesalpino's multilayered botany: metaphysics, classification, therapeutics and plant cultivation**

Fabrizio Baldassarri

Villa I Tatti, Harvard University

e-mail: [fabrizio.baldassarri@unipd.it](mailto:fabrizio.baldassarri@unipd.it)

Keywords: Renaissance botany, plant philosophy, herbaria, plant cultivation

Andrea Cesalpino is generally known as a sort of father of systematics in botanical knowledge, thanks to his organization of plants according to a binomial way that anticipated—and was praised by—Carl Linnaeus. Yet, besides this issue, the figure of Cesalpino presents several different aspects, making him a more central figure to understand and contextualize sixteenth-century botany at large. In this presentation, I aim to outline Cesalpino's multilayered attention to plant studies, highlighting the crucial features shaping Renaissance construction of botany.

## **Ambiregnal chaos: The diversity and taxonomy of protists**

Ivan Čepička

Department of Zoology, Charles University, Prague, Czech Republic

e-mail: [ivan.cepicka@natur.cuni.cz](mailto:ivan.cepicka@natur.cuni.cz)

Keywords: protist, taxonomy, diversity, phylogeny, evolution

The definition of protists has long been debated among protistologists, with no single variant universally accepted. Here, I will adhere to the definition that protists encompass all eukaryotes excluding animals (Metazoa), land plants (Embryopsida), and fungi (Fungi). Protists represent the vast majority of the "deep" eukaryotic diversity and play a crucial role in understanding eukaryogenesis. Due to the large diversity of the protists, the traditional concept of eukaryotic kingdoms was replaced by the concept of supergroups approximately 20 years ago, and Linnean ranks are not always applied to higher protist taxa. Despite the recognition of protists' distinct nature over 150 years ago, they are still routinely categorized as either "animals" or "plants" (or a combination of both). This has led to the separate fields of protozoology ("animal-like" protists) and algology (photosynthetic protists), even though neither group is monophyletic. This duality is further emphasized by the fact that the taxonomy of "protozoa" follows the zoological nomenclature code (ICZN), while that of "algae" follows the botanical one (ICN). In my talk, I will provide a comprehensive overview of recent changes in protist (eukaryotic) phylogeny and taxonomy and their implications for our understanding of eukaryotic evolution.

## **The modern face of taxonomy: bridging biodiversity, technology, and society**

Pablo Muñoz-Rodríguez

Department of Biodiversity, Ecology and Evolution, Faculty of Biological Sciences. Universidad Complutense de Madrid, Spain

e-mail: [pablo.munoz@ucm.es](mailto:pablo.munoz@ucm.es)

Keywords: taxonomy, integration, technology, monographs, impact

Taxonomy is the foundation of biodiversity science, yet it remains marginalised in an era dominated by big data and high-throughput biodiversity research. This talk will explore how modern taxonomy must evolve to remain central: integrative, collaborative, fast, and digitally accessible. Drawing on ongoing global studies of two megadiverse genera, *Ipomoea* and *Acalypha*, I will argue that taxonomists—whose primary and essential task is to describe species and classify the world's diversity—must also take the lead in interdisciplinary efforts that inform conservation, evolution, and policy. Technological advances (genomics, AI, digital platforms) offer unprecedented tools, but they depend on robust taxonomic frameworks to be effective and meaningful. Taxonomic monographs, far from outdated, provide an ideal structure to integrate these technologies with comprehensive taxonomic knowledge, becoming dynamic, long-lasting resources rooted in evolutionary thinking and supported by open data. In doing so, they allow taxonomy to extend its reach into fields from climate change to food security. Ensuring visibility, completeness, and timely publication is critical if our work is to be impactful. To face the biodiversity crisis, we must reclaim taxonomy's role not as a supporting science, but as a driver of knowledge, relevance, and impact across society.

## **Turrill's Omega: an innovative index for solving species concept issues**

1. Lorenzo Peruzzi, 2. Jacopo Franzoni, 3. Antonio Giacò

PLANTSEED Lab, Department of Biology, University of Pisa, Italy

e-mails: 1. [lorenzo.peruzzi@unipi.it](mailto:lorenzo.peruzzi@unipi.it), 2. [jacopo.franzoni@biologia.unipi.it](mailto:jacopo.franzoni@biologia.unipi.it), 3. [antonio.giaco@biologia.unipi.it](mailto:antonio.giaco@biologia.unipi.it)

Keywords: integrative taxonomy, taxonomic hypotheses, taxonomy, species concepts, systematics

Biological research at all levels fully relies on the use of taxa, expressed in the form of a Latin binomial for species. These are often assumed to be facts, but they are hypotheses on the distribution of biological variation in nature that can change when new systematic knowledge is produced. The long-standing scientific problem affecting systematics and taxonomy, but also having deep impacts on other biological disciplines, is that the binomial itself does not bring the information concerning its solidity as a taxonomic hypothesis. The project aims to provide a standardized index that allows to estimate the degree of support of a taxonomic hypothesis, based on the systematic data available in literature. The Turrill's Omega Index (TOI) is designed for vascular plants, the most important element of terrestrial habitats, whose systematic investigation is particularly challenging due to several biological phenomena such as hybridization, dysploidy, and polyploidy. TOI consists of a single number estimating the solidity of any taxonomic hypothesis, and is calculated from the integration of seven categories of systematic data: chemosystematics, cytogenetics, ecology, genealogy, geography, morphology, and reproductive biology. TOI will be a turning point in systematics and taxonomy, by providing a practical and reliable solution to the long-standing debate concerning species delimitation and species concepts.

# Oral presentations

## **Taxonomy and phylogeny of plagiopylean ciliates in South Yellow Sea**

1. Ran Li, 2. Xiaozhong Hu

Institute of Evolution and Marine Biodiversity, Ocean University of China

e-mails: 1. [244670471@qq.com](mailto:244670471@qq.com), 2. [xiaozhonghu@ouc.edu.cn](mailto:xiaozhonghu@ouc.edu.cn)

Keywords: Ciliophora, Plagiopylea, taxonomy, infraciliature, phylogeny

Plagiopylea is a class of obligate anaerobic ciliates inhabiting hypoxic or anoxic environments, often with prokaryotic symbionts. Despite their ecological significance and relevance to anaerobic adaptation studies, research on this group remains scarce, especially in China. To bridge this gap, we conducted a comprehensive survey in the coastal wetlands of the southern Yellow Sea, documenting 26 species across three families and nine genera. Key findings include: (1) establishment of three new genera in Sonderiidae based on oral morphology, (2) discovery and description of 12 new species, (3) detailed characterization of 11 poorly known species, providing their ciliary patterns for the first time and clarifying historical ambiguities, (4) a systematic review of 51 valid species, summarizing diagnostic features and providing identification keys for six non-monospecific genera, (5) phylogenetic analyses of 136 marker gene sequences from 33 species/populations, confirming the stable monophyly of Plagiopylidae and Trimyemidae while revealing Sonderiidae as paraphyletic. Integrating morphological and molecular data, we inferred the phylogenetic relationships and evolutionary trends within each family, contributing to a clearer understanding of Plagiopylea systematics and evolution.

## **Estimating the morphological evidence for taxonomic decisions: A Bayesian model-based approach**

1. Manuel Tiburtini<sup>1</sup>, 2. Luca Scrucca<sup>2</sup>, 3. Lorenzo Peruzzi<sup>1</sup>

<sup>1</sup>PLANTSEED Lab, Dipartimento di Biologia, Università di Pisa, Italy; <sup>2</sup>Dipartimento di Statistica, Università di Bologna, Italy

e-mails: 1. [manuel.tiburtini@biologia.unipi.it](mailto:manuel.tiburtini@biologia.unipi.it), 2. [luca.scrucca@unibo.it](mailto:luca.scrucca@unibo.it), 3. [lorenzo.peruzzi@unipi.it](mailto:lorenzo.peruzzi@unipi.it)

Keywords: species delimitation, morphometrics, Bayesian inference, Gaussian Mixture Models, taxonomic hypothesis testing

In no other science has there been as much empty verbiage about the meaning of a word as in taxonomy with the term species. The species problem reflects not only empirical ambiguity but also deep conceptual entanglements—between typological and population thinking, morphology and lineage, and the subjectivity inherent in deciding what we wish to call a “species”. Morphology is the sole visible attribute of a species, commonly interpreted as continuous variation forced into discrete taxonomic categories. While phylogenetic methods have developed sophisticated statistical frameworks to test species circumscriptions, morphological data are still largely evaluated qualitatively or through techniques lacking biological realism and statistical rigour. By treating morphometric data as generated by a mixture of multivariate Gaussian distributions and species as latent statistical classes, we can quantify the evidence in support of a taxonomic hypothesis through a Bayesian model-based framework grounded in Gaussian Mixture Models (GMMs). This reframes the species problem as estimating the dimension of a model on morphological data and shows how morphological variation can be assessed through model evidence rather than subjective belief.

## **Next Generation Taxonomy of Ciliophora: a checkpoint after six years of experience**

1. Valentina Serra, 2. Giulio Petroni

Department of Biology, University of Pisa

Keywords: Ciliates, taxonomy, systematics, genomics, mitochondrial genome, phylogeny

e-mails: 1. [valentina.serra@unipi.it](mailto:valentina.serra@unipi.it), 2. [giulio.petroni@unipi.it](mailto:giulio.petroni@unipi.it)

The “NGTax” project (extended title: “Next Generation Taxonomy: Ciliophora and their bacterial symbionts as a proof of concept”) is a European RISE project (H2020-MSCA-RISE-2019) which aims to connect University’s and Company’s staff from different countries, towards mobility. The project is scientifically focused on integrative systematics of ciliates (Phylum Ciliophora) and of their bacterial symbionts including ecology and genomics. The project proposes the integration in taxonomy of the most advanced genomics and bioinformatics techniques, taking into account also the holobiont concept: we named this approach Next Generation Taxonomy (NGTax). The NGTax workflow proposes a new approach to modern taxonomy aimed to integrate the holobiont concept with genomic and bioinformatics analyses together with classical/morphological tools traditionally used in taxonomy. Six years after the start of the project and the use of the NGTax workflow, this presentation will explore the results achieved through a panorama of selected case studies.



## **New Artificial Intelligence approach towards digitally extended herbarium specimens**

1. Stefano Martellos, 2. Weiwei Liu

Department of Life Sciences, University of Trieste

e-mails: 1. [martelst@units.it](mailto:martelst@units.it), 2. [weiwei.liu@phd.units.it](mailto:weiwei.liu@phd.units.it)

Keywords: computer vision, metadata, model, natural history collection

Artificial Intelligence (AI) is part of our daily life, and its application is fundamental in several fields. However, as far as digitization and digital extension of Natural History Collection (NHC) specimens are concerned, its exploitation is still at the beginning. AI can support digitization efforts especially addressing their most relevant bottleneck, i.e. the transcription of metadata which are present on the label(s) of a specimen. Currently, this task requires relevant amounts of human labor both in small scale and industrial approaches to digitization. The introduction of AI supported label(s) transcription tools would dramatically increase the efficiency of any digitization workflow. The major obstacle at the moment is the training of AI models, which requires large training pools (images + verbatim transcribed metadata, i.e. “ground truth”). AI could also be helpful for extending specimens, linking them to other information than the transcriptions of the labels. An AI model could be trained to identify the writing style on a label and associate one specimen with others collected by the same person, or to other documents. Another example could be the use of AI for morphometric measures, and to associate a specimen with others with similar features. This contribution aims at highlighting the most interesting AI approaches in the digitization and digital extension of NHC specimens, and to provide ideas on other possible research in the field.

## **Presentation of taxonomic monograph of hyalospheniid testate amoebae (Amoebozoa)**

1. Anush Kosakyan<sup>1,2,3,4,5</sup>, 2. Ralf Meisterfeld<sup>6</sup>, Enrique Lara<sup>7</sup>, 3. Clement Duckert<sup>1</sup>, 4. Edward A.D. Mitchell<sup>1</sup>

<sup>1</sup>Laboratory of Soil Biodiversity, University of Neuchâtel, Switzerland; <sup>2</sup>Laboratory of Evolutionary Protistology, Institute of Biosciences, University of Sao Paulo, Brazil; <sup>3</sup>Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Ceke Budejovice, Czech Republic; <sup>4</sup>Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy; <sup>5</sup>National Biodiversity Future Center (NBFC), Palermo, Italy; <sup>6</sup>Institute for Biology II (Zoology), RWTH Aachen University, Germany; <sup>7</sup>Real Jardin Botanico, CSIC, Madrid, Spain

e-mails: 1. [anush.kosakyan@unimore.it](mailto:anush.kosakyan@unimore.it), 2. [meisterfeld@rwth-aachen.de](mailto:meisterfeld@rwth-aachen.de), 3. [enrique.lara@rjb.csic.es](mailto:enrique.lara@rjb.csic.es), 4. [clement.duckert@unine.ch](mailto:clement.duckert@unine.ch), 5. [edward.mitchell@unine.ch](mailto:edward.mitchell@unine.ch)

Keywords: testate amoebae, monograph, taxonomy, microbial eukaryotes

Testate amoebae are unicellular amoeboid eukaryotes enclosed in a shell which can be preserved for millions of years making them great bioindicators. Research on testate amoebae has primarily focused on environmental monitoring and palaeoecology; however later expanded to microbial ecology, forensic sciences, and ecotoxicology. Among testate amoebae, hyalospheniids represent one of the most conspicuous groups. Since G. Deflandre published his monograph on the genus *Nebela* in 1936 no systematic revision of this remarkable group of amoebae had been undertaken. In the meantime significant taxonomic advancements have occurred, leading to the description of numerous new species. A taxonomic revision of the group was clearly overdue. Here, we present a taxonomic revision of the infraorder Hyalospheniformes. A total of 14 genera, and 97 species are presented in detail. The monograph includes taxonomic keys; taxon descriptions; ecological, geographical, and taxonomical notes; annotated lists of doubtful and incertae sedis species; a list of synonyms; notes on molecular data; and light and scanning electron microscopic pictures, and/or original line drawings for each species. This book is designed to benefit of protistologists in general, and particularly ecologists and palaeoecologists, by assisting with the ease and accuracy of species identification, also serving as a valuable basis for future work regarding the taxonomy, biogeography, and ecology of these organisms.

## **Integrative systematics of a species-rich group of wild Mediterranean carnations (*Dianthus virgineus* L. complex, Caryophyllaceae)**

1. Jacopo Franzoni<sup>1</sup>, 2. Gianluigi Bacchetta<sup>2</sup>, 3. Fabio Conti<sup>3</sup>, 4. Gianniantonio Domina<sup>4</sup>, 5. Simone Fior<sup>5</sup>, 6. Luigi Minuto<sup>6</sup>, 7. Lorenzo Peruzzi<sup>1</sup>

<sup>1</sup>Department of Biology, University of Pisa, Italy; <sup>2</sup>Department of Life and Environmental Sciences, University of Cagliari, Italy; <sup>3</sup>School of Biosciences and Veterinary Medicine, University of Camerino, Italy; <sup>4</sup>Agricultural, Food and Forestry Sciences, University of Palermo, Italy; <sup>5</sup>Department of Environmental Systems Science, ETH Zürich, Switzerland; <sup>6</sup>Department of Earth, Environmental and Life Sciences, University of Genova, Italy  
e-mails: 1. [jacopo.franzoni@biologia.unipi.it](mailto:jacopo.franzoni@biologia.unipi.it), [lorenzo.peruzzi@unipi.it](mailto:lorenzo.peruzzi@unipi.it), 2. [bacchet@unica.it](mailto:bacchet@unica.it), 3. [fabio.conti@unicam.it](mailto:fabio.conti@unicam.it), 4. [gianniantonio.domina@unipa.it](mailto:gianniantonio.domina@unipa.it), 5. [simone.fior@usys.ethz.ch](mailto:simone.fior@usys.ethz.ch), 6. [luigi.minuto@unige.it](mailto:luigi.minuto@unige.it)

Keywords: *Dianthus*, genomics, karyology, Mediterranean, morphometrics

The *Dianthus virgineus* complex includes 21 putative wild carnations taxa in the central Mediterranean that were described only according to qualitative morphology, however their boundaries remain untested with other sources of biological evidence. Thus, we collected morphometric, cytogenetic, and genomic (ddRAD-seq) data from over 100 populations to test the current taxonomy of the complex. Morphometric clustering employing Gaussian Mixture Models identifies 10 groups, rarely matching current taxa or geography. A two-group hypothesis, geographically based, is better supported than the current taxonomy, with Sardinian, Sicilian, and Tunisian populations showing longer calyx teeth and mucros than French, Italian, Corsican, and Tuscan Archipelago ones. All populations are diploid ( $2n = 30$ ) and genome size follows geography but not taxonomy, with relatively larger genomes in Corsican, Sardinian, and Sicilian populations. Genomic data reveal a single lineage in the central Mediterranean, with two intersecting genetic clines: one running from southern France through the Italian Peninsula, and one involving populations from Tuscany, Corsica, Sardinia, Tunisia and Sicily. Genetic differentiation among populations was significant, fitting well with an isolation-by-distance model. Overall, our results suggest that the numerous currently accepted taxa are not supported, and only one species with two geographically vicariant, widely distributed subspecies should be recognized.

## **Review of the taxonomic history of genus *Lacrymaria* with production of a morphological identification key**

1. Alessandro Allievi, 2. Giulio Petroni, 3. Valentina Serra

Department of Biology, University of Pisa

e-mails: 1. [a.allievi@phd.unipi.it](mailto:a.allievi@phd.unipi.it), 2. [giulio.petroni@unipi.it](mailto:giulio.petroni@unipi.it), 3. [valentina.serra@unipi.it](mailto:valentina.serra@unipi.it)

Keywords: taxonomy, systematics, identification, Ciliophora, *Lacrymaria*

The taxonomic history of the ciliate genera *Lacrymaria* Bory de St. Vincent, 1824 and *Phialina* Bory de St. Vincent, 1824 is long and complex, during which genus *Phialina* was merged into *Lacrymaria* by Alfred Kahl (1930), who abolished the past discriminant based on cytostome location, and separated again by Wilhelm Foissner (1983), who introduced a new one based on the presence of a contractile neck. Now, recent molecular studies show that the two genera are monophyletic collectively but not separately (Rajter et al., 2019; Allievi et al., in preparation), which suggests they might have to be combined again, presumably under name *Lacrymaria* after Kahl's precedent. These genera include many species highly morphologically diverse, often observed and/or described rarely or just once. This study takes the form of a research, compilation, translation, and comparison of all species descriptions under either name between 1773 and 2023, with a fivefold aim: 1) check systematically all such descriptions noting opportunities for lumping and splitting in future studies; 2) synthesize the taxonomic history of this genus; 3) propose to re-assign all species of current genus *Phialina* to *Lacrymaria*; 4) provide an extensive morphology-based guide to the identification of all *Lacrymaria* species; 5) make available the original description of each in the original authors' own words.

## **The Botanic Garden of Pisa under Cesalpino's direction: a historical snapshot of 16th-century Botany**

1. Giovanni Astuti<sup>1</sup>, 2. Marco D'Antraccoli<sup>1</sup>, 3. Francesco Roma-Marzio<sup>1</sup>, 4. Roberta Vangelisti<sup>1</sup>, 5. Lorenzo Peruzzi<sup>1,2</sup>

<sup>1</sup>Orto e Museo Botanico, University of Pisa, Italy; <sup>2</sup>PLANTSEED Lab, Department of Biology, University of Pisa, Italy

e-mails: 1. [giovanni.astuti@unipi.it](mailto:giovanni.astuti@unipi.it), 2. [marco.dantraccoli@unipi.it](mailto:marco.dantraccoli@unipi.it), 3. [francesco.romamarzio@unipi.it](mailto:francesco.romamarzio@unipi.it), 4. [roberta.vangelisti@unipi.it](mailto:roberta.vangelisti@unipi.it), 5. [lorenzo.peruzzi@unipi.it](mailto:lorenzo.peruzzi@unipi.it)

Keywords: Aldrovandi, America, catalogues, herbaria, medicinal plants

The world's oldest university botanical garden, located in Pisa, was founded in 1543 by Luca Ghini. In 1563, the garden was relocated to a second site under the direction of Andrea Cesalpino, a former student of Ghini, and in 1591, it was moved to its final and current location. Lists of plants cultivated in the garden's first location had been compiled by Ulisse Aldrovandi, another of Ghini's students, starting in 1549. A list of plants grown at the second site was compiled again by Aldrovandi in 1569, during one of the least documented periods in the garden's history. We studied this last list, to determine how many and which species were present and to gain broader insights into the garden's role at the time. To translate the recorded names into current taxonomy, we searched for the names used in the list in the herbarium specimens of both Aldrovandi and Cesalpino. We were able to identify 284 names at the species level, out of the 336 listed in the manuscript. However, 24 names were redundant, resulting in a final total of 260 distinct species. Additionally, 16 names were identified at least at the genus level, and 11 at least at the family level. For 6 names, no identification was possible. The list clearly reflects the garden's strong focus on medicinal plants, which were the main interest of botanists at the time. Notably, most of the species are native to the area around Pisa or nearby, although a few alien species from distant regions (i.e. America) were also present.

## Progress in taxonomy and phylogeny of freshwater pleurostomatid ciliates

1. Gongao Zhang<sup>1,2</sup>, 2. Weibo Song<sup>1,2</sup>

<sup>1</sup>Institute of Evolution & Marine Biodiversity, Ocean University of China; <sup>2</sup>MOE Key Laboratory of Evolution & Marine Biodiversity, Ocean University of China

e-mails: 1. [zgat2009@163.com](mailto:zgat2009@163.com); [wsong@ouc.edu.cn](mailto:wsong@ouc.edu.cn)

Keywords: Pleurostomatids, wetland, phylogeny, taxonomy

Pleurostomatid ciliates are highly recognizable due to their laterally compressed body shape, a slit-like cytostome positioned along the ventral margin, and short, bristle-like cilia on the left side. This group is widely distributed and play a crucial role in the ecosystem. In recent years, the author has conducted a comprehensive morphological and systematic investigation of pleurostomatids in the Lake Weishan wetland. The key findings of this research include: (1) phylogenetic analysis and reclassification: based on the morphological characters, this group has been divided into two suborders: Amphileptina Jankowski, 1967 and Protolitonotina Zhang et al., 2024. The newly established suborder Protolitonotina represents the earliest diverging lineage within the order; (2) establishment of a new genus: *Heterolitonotus* Zhang et al., 2024 has been identified and described; (3) discovery of a new parasitic species: *Apoamphileptus paraclaparedii* Zhang et al., 2024 has been described. Phylogenetic analysis reveals that *Apoamphileptus* shares a close evolutionary relationship with *Pseudoamphileptus* Foissner, 1983 within the family Amphileptidae Bütschli, 1889. This study provides essential new insights into the diversity, taxonomy, and phylogeny of freshwater pleurostomatids, significantly enhancing the understanding of this ecologically important ciliate group.

## **Systematics and taxonomy of the Italian endemic *Centaurea aplolepa* (Asteraceae): from nomenclature to population genetics**

1. Antonio Giacò<sup>1</sup>, 2. Mario Pentassuglia<sup>1</sup>, 3. Ludovica Fittipaldi<sup>2</sup>, 4. Andrea Coppi<sup>2</sup>, 5. Lorenzo Peruzzi<sup>1</sup>

<sup>1</sup>Department of Biology, University of Pisa, Italy; <sup>2</sup>Department of Biology, University of Florence, Italy

e-mails: 1. [antonio.giaco@biologia.unipi.it](mailto:antonio.giaco@biologia.unipi.it), 2. [m.pentassuglia3@studenti.unipi.it](mailto:m.pentassuglia3@studenti.unipi.it), 3. [ludovica.fittipaldi@edu.unifi.it](mailto:ludovica.fittipaldi@edu.unifi.it), 4. [andrea.coppi@unifi.it](mailto:andrea.coppi@unifi.it), 5. [lorenzo.peruzzi@unipi.it](mailto:lorenzo.peruzzi@unipi.it)

Keywords: Mediterranean, endemism, allopatry, morphometrics, cytogenetics

*Centaurea* L., with ca. 600 species, is considered one of the most challenging of the Mediterranean genera under both systematic and taxonomic perspectives. Within this genus, the Italian endemic *C. aplolepa* Moretti, with ten subspecies distributed mostly in Tuscany and Liguria, is still waiting for a modern taxonomic revision. To clarify the systematic relationships within this species and to update the current taxonomic circumscription, considered artificial by several authors, a systematic and taxonomic investigation has been conducted. In a nomenclatural revision, we fixed the application of 17 names within *C. aplolepa* and *C. leucophaea* Jord., a geographically and phylogenetically closely related species. During summers 2023 and 2024, we sampled 18 populations of *C. aplolepa* and six populations of related taxa (*C. aetaliae* (Sommier) Bég., *C. leucophaea*, *C. ilvensis* (Sommier) Arrigoni, and *C. paniculata* L.). Chromosome counts confirmed the diploid status for all sampled populations. Then, morphometric analyses and population genetics with AFLP allowed the recognition of seven allopatric groups, possibly originated through isolation and diversification during the Quaternary climatic oscillations. From a taxonomic perspective, our proposal is to consider each of the seven groups as distinct species. These latter include: one species (*C. aetaliae*) endemic to the Elba Island (Tuscany), *C. paniculata*, and five species that were previously considered as subspecies within *C. aplolepa*.

## Pisa's botanical garden pond: a hidden gem of Gastrotricha biodiversity

1. Francesco Saponi<sup>1,2,3</sup>, 2. Agata Cesaretti<sup>3</sup>, 3. Anush Kosakyan<sup>2,3</sup>, 4. Valentina Serra<sup>4</sup>, 5. M. Antonio Todaro<sup>3</sup>

<sup>1</sup>Dipartimento di Scienze della Terra e del Mare, Università degli Studi di Palermo, Italy; <sup>2</sup>NBFC, Palermo, Italy;

<sup>3</sup>Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia, Modena, Italy; <sup>4</sup>Dipartimento di Biologia, Università di Pisa, Italy

e-mails: 1 [francesco.saponi@unimore.it](mailto:francesco.saponi@unimore.it), 2. [agata.cesaretti@unimore.it](mailto:agata.cesaretti@unimore.it), 3. [anush.kosakyan@unimore.it](mailto:anush.kosakyan@unimore.it), 4. [valentina.serra@unipi.it](mailto:valentina.serra@unipi.it), 5. [antonio.todaro@unimore.it](mailto:antonio.todaro@unimore.it)

Keywords: Pisa, garden, Gastrotricha, diversity

Gastrotricha is a phylum of microscopic aquatic animals that includes over 900 species, with 375 of them residing in freshwater environments. Because of their rapid growth and ability to reproduce parthenogenetically, these creatures can quickly establish stable populations in any water body, starting from just one adult or a single egg. In fact, gastrotrichs can be found in nearly every aquatic or limno-terrestrial habitat, whether natural or artificial. Ponds in botanical gardens are man-made habitats that support diverse microfauna, including taxa that are new to science. The introduction of animals through plants, water, and sediment replacement, together with the variety of niches they accommodate, improve their natural ability to host microscopic organisms. In this study, we aim to improve the knowledge of gastrotrichs diversity in these habitats, describing two new interesting species found in the main pond of Pisa's botanical garden. The first, benthonic, shows some peculiar traits that allow its inclusion in the *Chaetonotus macrolepidotus* species group, while the second, planktonic, belongs to the rare genus *Setopus*. Both these taxa lack of any molecular data, for this reason the COI sequences here provided may be a key contribution to the resolution of the puzzling phylogeny of the Oiorpata group. The finding of gastrotrichs adopting two different lifestyles further emphasises the importance of this type of biotope for these organisms.



## Challenges of taxonomically complex groups: lessons from the genus *Thymus* (Lamiaceae) and subfam. Carduoideae (Asteraceae)

1. Svetlana Banchева<sup>1,2</sup>, 2. Ina Aneva<sup>2</sup>, 3. Petar Zhelev<sup>3</sup>, 4. Dimitar Ivanov<sup>2</sup>

<sup>1</sup>Botanical Garden of the Bulgarian Academy of Sciences, Sofia, Bulgaria; <sup>2</sup>Institute of Biodiversity and Ecosystem Research Bulgarian Academy of Sciences, Sofia, Bulgaria; <sup>3</sup>Department of Dendrology, University of Forestry, Sofia, Bulgaria

e-mails: 1. [sbancheva@yahoo.com](mailto:sbancheva@yahoo.com), 2. [ina.aneva@abv.bg](mailto:ina.aneva@abv.bg), 3. [petar.zhelev@ltu.bg](mailto:petar.zhelev@ltu.bg)

Keywords: *Thymus*, Carduoideae, DNA barcoding, secondary metabolites, species delimitation

The genus *Thymus* (Lamiaceae) and subfam. Carduoideae (Asteraceae) represent classic examples of taxonomically complex groups (TCGs), characterized by intricate evolutionary relationships, hybridization, overlapping morphological variability, and unresolved species boundaries. These features present significant challenges for accurate taxonomic and phylogenetic resolution. To address these complexities, a multidisciplinary approach is required, combining classical methodologies with modern molecular tools and chemotaxonomy. Our studies highlight a high level of genetic diversity within *Thymus*. The analysis of secondary metabolites in species from *T.* sections *Serpyllum* and *Hyphodromi* reveals distinct interspecific and inter-sectional differentiation. These findings highlight the importance of phytochemical studies as a valuable tool for understanding species boundaries and evolutionary relationships within taxonomically complex plant groups. They also have important practical significance, as they can be used to create high-quality *Thymus* products. As a result of the large-scale comparative study of the subfamily Carduoideae, it was established that 148 species and 19 subspecies, belonging to 21 genera, occur on the territory of Bulgaria. Fifteen new species and seven subspecies have been identified for the Bulgarian flora, compared to the previous most up-to-date information.

## **Optimizing scientific illustration: advancements in GNU-based techniques**

Giuseppe Montesanto

Museum of Natural History, University of Pisa, Italy

e-mail: [giuseppe.montesanto@unipi.it](mailto:giuseppe.montesanto@unipi.it)

Keywords: invertebrates, taxonomy, GIMP, digital inking, Oniscidea

Scientific illustration is critical to biological systematics and animal taxonomy, and can depict complex morphologies or anatomical features, vital for species identification. Digital methodologies enable the visual comparison of distinctive characteristics among taxa, emphasizing specific details, particularly when expensive instrumentation is unavailable. Furthermore, illustrations show a representation of characters that would require multiple focal planes under the microscope. The present work is an update of two papers on digital inking techniques. This GNU-methodology has been used for several animal taxa over the last 10 years, leading to the description of new species. After a decade of research, including the release of new software versions and techniques, the work has now been actualized. The implementation of a faster method for drawing setae and hairy areas is of particular interest. In addition, new methods for digital stippling have been added. The latter method is the most time-consuming approach for individuals who are about to incorporate three-dimensional elements into their drawings or to render light and dark by adding highlights and shadows to a drawing that consists primarily of monochromatic patterns. The imminent publication of OniscidBase (the online world catalogue of terrestrial isopods) will facilitate the study of biodiversity also for non-specialists by providing illustrations of terrestrial isopods, mainly for little-known species.

## **Comprehensive approach to a taxonomically complex group – genus *Quercus* (Oak) in the Bulgarian flora**

1. Petar Zhelev<sup>1</sup>, 2. Ina Aneva<sup>2</sup>, 3. Evgeni Tsavkov<sup>1</sup>, 4. Nikolay Tsvetanov<sup>1</sup>

<sup>1</sup>Department of Dendrology, University of Forestry, Sofia, Bulgaria; <sup>2</sup>Department of Plant and Fungal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

e-mails: 1. [petar.zhelev@itu.bg](mailto:petar.zhelev@itu.bg), 2. [ina.aneva@abv.bg](mailto:ina.aneva@abv.bg)

Keywords: oaks, taxonomy, DNA barcoding, morphology, morphometry

The genus *Quercus* on the Balkans and particularly in Bulgaria is characterized by great diversity and complexity. It includes many taxa, some of them with controversial status, with extensive hybridization and overlapping of phenotypic traits between the taxa. As a result, genus *Quercus* is considered a complex taxonomic group (TCG) and the approach to TCGs must be different than the traditionally applied. The last synthesis of the oaks' taxonomy in Bulgaria was published in 1966. Considering the development of the concepts and methods used in plant systematics during the last decades, there is an urgent need of rethinking and updating of the taxonomic scheme of the genus in Bulgaria. Here we present first results of implementation a combined approach in attempt to resolve the taxonomic puzzle of Bulgarian oaks. DNA barcoding based on one nuclear (ITS) and two chloroplast markers (trnH-psbA, matK), together with a combination of these markers, was promising but not sufficient, as none of the markers proved to be species-specific. Our approach includes combining several methods and markers: DNA barcoding, traditional morphology and morphometry, geometric morphometry, leaf epidermis morphology studied by scanning electron microscopy, distribution of genetic diversity within and among species and populations, and metabolomics. We believe the synergy among the methods applied could bring valuable insight into the taxonomy and evolution of the taxonomically complex *Quercus* group.

## **Systematic revision of the genus *Pericephalus* Budde-Lund, 1909 with a case study of tropical species trade (Crustacea, Isopoda, Oniscidea)**

1. Stefano Taiti<sup>1</sup>, 2. Giuseppe Montesanto<sup>2</sup>

<sup>1</sup>Research Institute on Terrestrial Ecosystems, National Research Council, Florence, Italy; <sup>2</sup>Natural History Museum, University of Pisa, Italy

e-mails: 1. [stefano.taiti@cnr.it](mailto:stefano.taiti@cnr.it), 2. [giuseppe.montesanto@unipi.it](mailto:giuseppe.montesanto@unipi.it)

Keywords: Armadillidae, new species, Oriental Region, isopod trade

At present, the suborder Oniscidea (Crustacea, Isopoda) comprises ca. 4,100 species in 568 genera and 39 families. Armadillidae is one of the largest families with 84 genera and 732 species, distributed mainly in tropical countries. Some genera are still poorly known due to old and outmoded descriptions dating back to the beginning of last century. One of these is the genus *Pericephalus* Budde-Lund, 1909, which comprises two already known species, i.e., *Pericephalus feae* (Budde-Lund, 1895) from Myanmar and *P. marcidus* (Budde-Lund, 1904) from Malaysia, plus four new species, i.e., *Pericephalus* n. sp. 1 from Malaysia and *Pericephalus* new sp. 2, 3, and 4 from Thailand (present work). The genus is characterized by conglobating ability, cephalon with a wide frontal shield, large ventral lobes on epimera of pereonites 1 and 2, hourglass-shaped telson, and pleopods with polyspiracular lungs. Due to its large size (15 mm) and its yellow and grey-brown colour, *Pericephalus* n. sp. 2 from Thailand is one of many species of terrestrial isopods, mainly from tropical and Mediterranean areas, that is used in the pet trade. In the last two decades, woodlice have become very popular among hobbyists thanks to their easy care, beautiful colours, and minimal space requirement. Nowadays, many live specimens, often belonging to species not yet described and with fictitious names, are sold around the world. This trade is not regulated yet and in the future may cause several problems, such as threatening native populations and the likelihood of alien species invading new regions.

## Comprehensive study on the diversity, distribution, phytochemical profiles, and genetic variability of *Thymus* in Bulgaria: challenges and perspectives

1. Ina Aneva<sup>1</sup>, 2. Dimiter Ivanov<sup>1</sup>, 3. Ekaterina Kozuharova<sup>2</sup>, 4. Svetlana Nikolova<sup>1,3</sup>, 5. Vladimir Vladimirov<sup>1</sup>, 6. Petar Zhelev<sup>4</sup>

<sup>1</sup>Department of Plant and Fungal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria; <sup>2</sup>Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, Bulgaria; <sup>3</sup>Botanical Garden, Bulgarian Academy of Sciences, Sofia, Bulgaria;

<sup>4</sup>Department of Dendrology, University of Forestry, Sofia, Bulgaria

e-mails: 1. [ina.aneva@abv.bg](mailto:ina.aneva@abv.bg), 2. [ina\\_kozuharova@yahoo.co.uk](mailto:ina_kozuharova@yahoo.co.uk), 4. [petar.zhelev@ltu.bg](mailto:petar.zhelev@ltu.bg)

Keywords: *Thymus*, taxonomy, phytochemistry, genetic diversity, sustainable use

The genus *Thymus* in Bulgaria includes 21 species (two national endemics and four Balkan endemics) valued for their medicinal, culinary, and aromatic uses. However, accurate species identification and sustainable use remain limited. This study presents the first integrated analysis of *Thymus* diversity in Bulgaria, combining floristic, phytochemical, and genetic data. Fieldwork across 20 regions documented 283 populations and mapped distributions of conservation-sensitive taxa (e.g. *T. perinicus*, *T. stojanovii*). About half of the species are morphologically discrete (“good species” such as *T. atticus*), while the rest form hybridizing continua, complicating taxonomy and chemical profiling. Eleven species were genetically analyzed using trnH-psbA, matK, and ITS2 markers, revealing eight haplotypes and taxonomic inconsistencies. Morphological variability and hybridization hinder classification and chemical profiling. Based on their distinct chemotypes, *T. longedentatus*, *T. zygioides*, and *T. pannonicus* were selected for cultivation at the BAS Botanical Garden, producing standardized thyme teas. This multidisciplinary framework lays the foundation for high-value medicinal plant production in Southeast Europe, with implications for biodiversity conservation, species authentication and sustainable resource use.