Consensus on future research directions in the Phylum Rotifera

Running Head: Global priorities in rotifer research

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Abstract

Rotifers play key roles in aquatic ecosystems, yet significant uncertainty remains about their diversity and evolution, and basic knowledge is still lacking to address practical challenges related to global change. To identify the major knowledge gaps hindering progress, we carried out a Delphi process both online and during the 17th International Rotifer Symposium, involving more than forty experts working across diverse regions and subdisciplines. A total of 133 research questions were screened for relevance and clarity, and reduced to 100 for online scoring. These were evaluated on a 1-to-10 priority scale, after which 67 questions that exceeded 50% agreement were advanced to an in-person workshop. Through structured discussions and round-table voting, participants identified gold, silver, and bronze priority questions, while also considering the feasibility to address them, resulting in a final consensus set of high-priority questions across basic, applied, and philosophical perspectives. The strong support for questions on taxonomic knowledge transfer, digital curation, and AI-assisted identification highlights the emergence of a methodological subfield that links classical taxonomy with modern computational tools. Likewise, the emphasis on improving genetic markers and connecting DNA sequences with traits shows that molecular research is now viewed as a foundational component of rotiferology. This synthesis provides the first community-driven roadmap for advancing rotifer research. By articulating shared priorities and clarifying persistent knowledge gaps − including the lack of reliable phylogenies, uneven global sampling, and limited hypothesis-driven work − it establishes a foundation for future collaborative projects, funding strategies, and cross-disciplinary integration.

Keywords: communities, evolution, diversity, methods, populations, rotifer research agenda

1.Introduction

Collaborative identification and prioritization of fundamental research questions has become an effective way to organize knowledge in complex scientific disciplines. A common framework for this process is the Delphi technique, which uses repeated rounds of structured questionnaires to gather, compare, and refine expert opinions. This approach is useful not only to build consensus but also to identify priorities, and expose areas where viewpoints diverge (Hasson et al. 2000). It has been used successfully in broad ecological and conservation contexts (Sutherland et al. 2012; Mukherjee et al. 2015) and in more focused fields including subterranean biology, invasion science and meiofauna research (Enders et al. 2020; Mammola et al. 2020; Martínez et al. 2025). By creating a roadmap, such exercises are particularly valuable for disciplines at an inflection point, where a long history of research is met with new technological capabilities and pressing environmental challenges, creating a timely opportunity to redefine research priorities.

The study of Rotifera, a phylum of microscopic metazoans, exemplifies a discipline where long-standing research intersects with emerging tools and new environmental challenges. With a rich, century-old history of taxonomic and ecological research, our understanding of rotifers has been profoundly shaped by advancements in microscopy, molecular biology, and ecological modeling (Lampert 1997; Gómez 2005; Serra et al. 2019; Gansfort et al. 2020). These ubiquitous organisms, inhabiting freshwater, saline, and semi-terrestrial ecosystems, play pivotal roles in nutrient cycling, energy transfer, and serve as bioindicators of environmental health (Arndt 1993; Fontaneto et al. 2006; Ejsmont-Karabin 2012; Wallace et al. 2021). Their diverse morphologies, feeding mechanisms, and life-history strategies, including the remarkable anhydrobiotic capabilities, obligate parthenogenesis, and horizontal gene transfer of bdelloid rotifers, make them a subject for fundamental biological inquiry (Ricci 1983; Melone and Ricci 1995; Ricci and Boschetti 2003; King et al. 2005; Gladyshev and Meselson 2008; Declerck and Papakostas 2017; Hespeels et al. 2023). However, important gaps in rotifer research remain. Geographic and taxonomic biases continue to limit our understanding of global biogeography and biodiversity patterns (Dumont 1983; Fontaneto et al. 2012). In addition, the widespread occurrence of cryptic species complicates estimates of species richness, distribution, and ecological roles (May and Wallace 2019; Wallace et al. 2024; Walczyńska et al. 2024). Against this backdrop, we here report the results of an exercise to identify the most important outstanding research questions related to rotifers.

This paper argues that the time is ripe for a concerted, community-driven effort to identify the fundamental questions that will guide the next era of rotifer research. The rapid pace of environmental change, coupled with the advent of powerful new research tools (e.g., eDNA, genomics: Mohl et al. 2025; AI-assisted taxonomy and ecology: Chen et al. 2023; Ienaga et al. 2024; Zhang et al. 2024), creates both an urgent need and an unprecedented opportunity to address long-standing questions in rotifer biology. By synthesizing the collective expertise of the international rotifer research community, we can move beyond a fragmented and specialized approach towards a more integrated and strategic research agenda. This paper, therefore, does not test specific hypotheses, but rather presents the outcome of a comprehensive Delphi process designed to elicit, refine, and prioritize a consensus-based list of key unanswered research questions in the field of Rotifera research.

We aimed to identify a consensus-based list of the major unanswered questions in Rotifera research that may guide and shape future work. Through this collective initiative, we hope to help students, researchers, and funding agencies in setting priorities, and to encourage collaboration across different areas of rotifer biology by highlighting shared interests and persistent challenges. Importantly, articulating fundamental questions also stimulates the development of new hypotheses, since questions define the boundaries of inquiry, while hypotheses provide testable explanations. By doing so, this effort seeks not only to outline directions for future research but also to reinforce the iterative cycle of questioning and hypothesis testing that drives scientific progress. Ultimately, we hope to inspire a new wave of integrated and impactful research that will deepen our understanding of this fascinating phylum and strengthen our ability to conserve and manage the freshwater ecosystems upon which we all depend.

2. Materials and Methods

We followed a structured approach based on the Delphi technique to gather and prioritize expert opinions on key unanswered questions in Rotifera research. It was designed to be iterative, involving multiple rounds and feedback of expert consultation, moving from a broad collection of questions to a refined, prioritized list reflecting the current challenges, emerging trends and future directions deemed most critical by the international rotifer research community. This was developed through both pre-symposium online forms and in-person discussions during the workshop “Emerging Consensus and Key Questions in Rotifera Research”, held at the 17th IRS in Rio de Janeiro (see Fig. 1 for the workflow). Since 1976, a triennial meeting, the International Rotifer Symposium (IRS) has gradually become a focal point for discussion and collaboration. The most recent meeting, the 17th IRS, was held in Rio de Janeiro, Brazil, from 4 – 8th August, 2025.

During Phase 1, experts were invited to submit three to five key research questions. Over 230 email invitations were sent to individuals selected based on their contributions to innovative, high-impact, and high-volume research on Rotifera, and their participation in the International Rotifer Symposium (IRS). In addition, the invitation was shared via the rotifer-family newsletter mailing list to broaden outreach. In total, thirty-seven experts responded. For each contributor, their region of work, study environment, education level, ethnicity, and gender were recorded (see Figure 2). There was also the option to not answer for each of the demographic questions. A total of 133 questions were formulated, and these were screened for duplicates and refined for clarity, consistency, and alignment with the predefined criteria of relevance and testability, resulting in a list of 100 questions (see Supplementary Document S1).

To avoid overly broad or vague topics, we focused on questions that could realistically be addressed by a small research team or through a limited set of funded projects. To achieve this, we adopted an established methodology based on Sutherland et al. (2011a, b), which emphasizes a rigorous, democratic, and transparent approach to identifying key research questions. Thus, an ideal question should either directly suggest a research design or be framed in a way that allows it to be translated into specific, testable research hypotheses, according to the following criteria:

(i) be answerable through a realistic research design;

(ii) address significant gaps in current knowledge;

(iii) have a factual answer that is not dependent on value judgments;

(iv) be of a spatial and temporal scale that is feasible for a research team to address;

(v) not be formulated as a broad or vague topic area;

(vi) avoid being answerable with "it depends";

(vii) not be framed as a yes/no question (e.g., avoid phrasing it like "Is Lecanidae more species-rich than Brachionidae?");

(viii) and, if related to impacts or interventions, clearly include a subject, an intervention, and a measurable outcome.

During Phase 2, the 100 questions were ranked through an online voting process, prior to the in-person workshop. In this stage, experts rated each question on a scale from 1 to 10, where 1–3 indicated low relevance, 4–6 intermediate relevance, 7–9 high relevance, and 10 very high priority. Participants were instructed to base their assessments primarily on their scientific knowledge and expertise rather than on personal interest, while acknowledging that complete objectivity may not be attainable. Based on the online voting results, 67 questions were selected according to a pre-established consensus threshold of 50% agreement on high or very high priority, and these were brought forward to the experts attending the 17th IRS. The prioritized research questions were subsequently categorized into six themes for in-person voting. These themes broadly reflect major ecological topics or methodological approaches. However, the boundaries between themes were approximate as many questions span more than one area (Sutherland et al. 2012). This was done because previous exercises had shown that sorting questions into themes too early might unintentionally discourage cross-cutting perspectives and novel combinations of ideas.

Phase 3 consisted of an in-person workshop. All participants received the voting results and were asked to reflect on the 67 most relevant questions prior to the meeting. However, changes in gender balance and participant characteristics were expected during the 17th IRS due to logistical and availability constraints not present in the online process. Participants were self-divided into seven groups of 5–10 experts, with two groups assigned to *Community and Diversity* because this theme contained the highest number of questions. Group leaders were chosen for the in-person discussions and asked to ensure that the process remained democratic, with all views respected. Within each group, experts were instructed to rank all questions as gold, silver, or bronze, without limits on each type. Then, the group selected two gold, one silver, and one bronze questions. Votes during the selection process of gold, silver, and bronze questions were used to understand group consensus across each theme. Final decisions were made by a show-of-hands vote, requiring a consensus threshold of 75% for the selection of gold, silver, and bronze questions. Questions that did not reach consensus were retained in the final record to acknowledge areas of expert disagreement and to reflect the current diversity of perspectives within rotifer ecology. Gold questions generally reflected broad research across interdisciplinary topics. Silver questions addressed important but more focused topics. Bronze questions captured research gaps that were potentially overlooked but still considered essential by the group. A final plenary session then determined the overall top-priority questions while also accounting for the feasibility to address these questions.

All analyses were performed using R Statistical Software (v 4.4.2; R Core Team 2026). Boxplots were used to summarize the distribution, central tendency, and variability of question scores by theme. We tested whether mean question scores differed among themes using Welch's ANOVA, which does not assume equal variances or balanced sample sizes. A Sankey diagram illustrates the flow and relative importance of questions across themes, subthemes and priority levels.

3. Results

3.1 Expert Panel

The expert panel (n = 37) was geographically diverse but predominantly based in Europe, followed by representation from South America and North America. Most participants specialized in freshwater ecosystems and were senior researchers with over ten years of experience, indicating strong disciplinary expertise, and there was limited early-career researcher input. Ethnic representation was primarily white, with lower participation from underrepresented groups, and the gender balance was slightly male-skewed (56.8% male, 40.5% female, 2.7% preferred not to say) (Fig. 2).

3.2 Priority questions

Across themes, Communities and Diversity included the largest number of high-priority questions (21 out of 35; 60% within this theme), consistent with it also having the largest number of submitted questions. Although it had the lowest absolute number of questions, Human Impacts and Global Change showed the highest within-theme proportion of high-priority classifications (5 out of 6; 83.3%). Methods also contained many high-priority questions (16 out of 23; 69.6%), reflecting strong consensus on the need for methodological advances. Notably, *Methods* included many high-priority questions, reflecting a strong consensus that methodological advances (especially molecular tools, integrative approaches, and digital identification) are urgently needed to support progress across all research areas. Several subthemes cluster strongly toward high priority, including *Trait Evolution & Plasticity*, *Species Interactions*, *Functional Diversity*, *Environmental Stressors*, *Molecular & Genetic Tools*, and *Biodiversity Patterns & Distribution*. In contrast, *Morphology*, *Monitoring & Assessment*, and *Communication* show a higher proportion of lower-priority classifications.

Consensus dynamics during Phase 3 varied across themes. Most themes (*Ecosystems and Functioning*, *Evolution and Ecology*, *Methods*, *Populations*) reached full agreement on gold, silver, and bronze questions. In contrast, *Communities and Diversity* showed internal disagreement: one subgroup did not reach consensus on a gold question, likely reflecting differences between monogonont-focused and bdelloid-focused researchers. The highest-scoring question from the online voting round (Q05-What are the mechanisms behind the extreme tolerance of bdelloid rotifers?) from *Ecosystems and Functioning* did not appear among the final gold, silver, or bronze selections in the in-person workshop. This discrepancy illustrates how expert-panel composition and in-person deliberation can shift perceived priorities. *Human Impacts and Global Change* had only gold consensus questions, partly due to the smaller number of questions generated in this theme and its broader conceptual diversity. Moreover, most gold questions fell into the high-feasibility quadrant (Fig. 5). Some questions, however, were judged high priority but low feasibility (e.g., Q32, Q71, Q53), indicating important topics that require substantial methodological or conceptual advances. Only Q26 was rated highly feasible despite being also considered of low priority, suggesting accessible research opportunities that are not considered urgent.

From the full set of questions submitted by experts (see demographic composition in Fig. 2), the 67 questions that reached at least 50% agreement on their relevance during Phase 2 are listed below by theme (Boxes 1-6), without implying rank. Mean scores (1–10 scale) varied among themes (Fig. 3). Figure 4 visualizes how these questions assigned to the six initial themes were redistributed according to their priority scores and then grouped into more specific subthemes for Phase 3. The highlighted portion of Boxes 1–6 presents the questions selected during Phase 3; these were evaluated for both relevance and feasibility, and their classification as gold, silver and bronze reached at least 75% agreement (see Fig. 5). Note that for some questions, there may already be some theoretical understanding, but empirical support for the theory is still lacking across taxonomic groups or contexts. Differences between mean scores were small and confidence intervals largely overlapped (Welch's ANOVA: $F_{5,26.977} = 1.17$, $p = 0.350$), indicating that all themes received similarly high evaluations.

For each of the the six themes (n = 100 questions), the share of high-priority questions were as: *Communities and Diversity* 60% (n=35); *Ecosystems and Functioning* 72.7% (n=11); *Evolution and Ecology* 58.3% (n=12); *Human Impacts and Global Change* 83.3% (n=6); *Methods* 69.6% (n=23); *Populations* 76.9% (n=13).

Communities and Diversity

Diversity within rotifer communities encompass taxonomic, functional and genetic dimensions, serving as indicators of environmental change and providing insights into ecological processes across space and time (Wallace 2002; Obertegger et al. 2011; Kuczynska-Kippen et al. 2021; Wallace et al. 2021). However, rotifers seem to be nearly ubiquitous, occurring in environments ranging from permanent systems to temporary habitats only lasting a few days, which makes it difficult to fully characterize the diversity and structure of their communities (Wallace 2006; Segers 2007). This vast array of habitats suggests that we take a different approach to understand their communities. We could, for example, (1) consider the relative importance of qualitative versus quantitative differences in the structure of rotifer communities, (2) analyze the distribution of the physical and metabolic traits that they possess across their habitats, and (3) evaluate their capacity for rapid evolution within the diversity of the environments in which they are found. Alongside the need for new approaches, several knowledge gaps persist. These include limited spatial and temporal coverage, biases on methodological approaches, scarce information on functional and genetic diversity, limited understanding of biotic interactions and responses to multiple stressors. These shortcomings hinder our ability to generalize patterns, compare studies across regions, and link community changes to ecosystem processes. Among the most pressing topics, experts emphasize the need for intergenerational transfer of taxonomic knowledge, ensuring the detection of cryptic diversity and production of comparable inventories (Q71, Box 1). Moving towards trait- and interaction-based frameworks is equally essential, as these processes underpin structure communities and ecosystem functioning (Q97), and how environmental stressors may reshape their communities (Q18) and affect their roles in ecosystem processes (Q26). Rotifer diversity research also has an interdisciplinary component, as patterns and processes observed in rotifers can inform broader questions in other fields of research, such as ecology and evolution. Similarly, the identification of reliable bioindicators and trait-based metrics (Q89) has direct relevance for ecosystem monitoring and management across aquatic habitats.

Box 1. Selected questions in the Community and Diversity theme after Phase 2. Ranked questions resulting from Phase 3 are highlighted in orange.

Q12. How can we interpret and resolve the concept of cosmopolitanism among rotifer species? (Gold)

Q18. What are the effects of multiple environmental stressors on rotifer development and community structure? (Gold)

Q71. What strategies can sustain intergenerational transfer of taxonomic expertise in rotifer research? (Gold)

Q97. What types of ecological interactions occur between rotifers and other organisms, and how do these interactions affect community structure and ecosystem functioning? (Gold)

Q26. What are the effects of specific environmental stressors (e.g., increased temperature, nutrient enrichment, or emerging contaminants) on rotifer community functional traits and their contribution to ecosystem processes in freshwater environments? (Silver)

Q89. Which rotifer species or trait-based groups serve as effective bioindicators in lakes and reservoirs, and which environmental gradients drive their patterns? (Silver)

Q22. Does global rotifer biodiversity reflect the biodiversity of species commonly used as indicators of environmental degradation? (Bronze)

Q91. Which quantifiable traits best represent rotifer functional diversity across diverse aquatic

habitats? (Bronze)
Q08. Why has the number of rotifer species recorded in Latin America remained low, and what factors (e.g., sampling bias, taxonomic effort) explain this pattern? **Q14**. How do taxonomic and functional diversity patterns of rotifer communities vary across environmental gradients and biogeographic regions, and what ecological or evolutionary factors explain these patterns? **Q14**. What is the most appropriate framework to analyze beta diversity in rotifers: species contributions (SCBD) or local contributions (LCBD)? **Q15**. What are the environmental drivers that shape beta and functional diversity of rotifers across different regions? **Q17**. How do rotifer communities respond to rapid environmental change compared to crustaceans like copepods and Daphnia? **Q23**. Which rotifer community metrics (e.g., diversity, dominance, trophic interactions) are most sensitive to early biodiversity loss caused by pond desiccation and habitat fragmentation? **Q29**. How does cryptic diversity in monogonont rotifers vary between molecular and morphological assessments, and what does this reveal about species richness? **Q30**. How many rotifer species likely exist globally, considering cryptic diversity and sampling gaps? **Q31**. How does the scale of cryptic diversity differ between monogonont and bdelloid rotifers, considering their reproductive and genetic systems? **Q39**. How does spatial connectivity influence rotifer species or genotype exchange across freshwater metacommunities, and how can mesocosm experiments simulate this?

Q50. How does environmental changes affect the expression of plastic traits in species complexes (e.g., *Brachionus calyciflorus*)?

Q90. Which rotifer taxonomic or functional groups respond most sensitively to water quality degradation in rivers and streams, and how can they serve as bioindicators?

Q94. How do anthropogenic actions alter rotifer functional diversity in streams and ponds?

Ecosystems and Functioning

Rotifers often dominate aquatic ecosystems in abundance and play a central role in nutrient cycling and energy transfer through microbial food webs. They connect detritus, bacteria, algae, and other microorganisms to higher consumers such as crustaceans, insect larvae, and small fishes. With their high reproductive capacity and short generation times, rotifers can form large populations in response to fluctuations in food availability, facilitating the efficient use of ephemeral or newly available resources (Walz 1987; Gilbert 2022). Their extensive morphological and functional diversity (Obertegger and Flaim 2015; Balkić et al. 2017; Obertegger and Wallace, 2023) allows them to inhabit a wide range of aquatic systems from temporary ponds to oligotrophic and eutrophic lakes, making them ideal models for studying ecosystem responses to disturbance. Functional approaches have improved our understanding of zooplankton ecology (Branco et al. 2023), including links between rotifer feeding guilds and land use in tropical streams (Bomfim et al. 2023). However, the integration of frameworks combining response and effect traits (Hébert et al. 2017) remains limited (Huỳnh et al. 2024), leaving several major questions open. Relevant knowledge gaps concern how environmental stressors and anthropogenic disturbances reshape rotifer functional traits, community structure, and trophic roles (Q21, Q87, Box 2), how short- and long-term climate variability influences community resilience (Q25), and how rotifers contribute to energy transfer in tropical food webs (Q86). However, without a joint implementation across spatial and temporal scales of response-and-effect trait frameworks that meaningfully connect rotifers to ecosystem functioning, these key questions will remain partly unanswered.

Box 2. Selected questions in the Ecosystems and Functioning theme after Phase 2. Ranked questions resulting from Phase 3 are highlighted in purple.

Q21. What are the effects of specific environmental stressors (e.g., temperature, nutrients, contaminants) on rotifer functional traits and their contribution to ecosystem processes? (Gold)

Q87. How do anthropogenic disturbance gradients shape the functional composition and trophic roles of rotifer communities in freshwater and transitional systems? (Gold)

Q25. To what extent does the structure of rotifer communities reflect short-term versus long-term climate-induced changes in small aquatic ecosystems? (Silver)

Q86. How do rotifers contribute to energy transfer and trophic dynamics in tropical stream food webs? (Bronze)

Q005. What are the mechanisms behind the extreme tolerance of bdelloid rotifers?

Q055. How does desiccation duration affect hatching success and development time in rotifer resting eggs with different life-history strategies?

Q56. What molecular and physiological mechanisms enable rotifer resting stages to survive desiccation, and how do these mechanisms differ among taxa from contrasting hydrological regimes?

Q57. Which morphological traits of rotifer resting eggs predict delayed hatching or reduced viability across environmental gradients (e.g. salinity, nutrients)?

Evolution and Ecology

Evolutionary aspects within a taxon of interest have been the focus of several lines of research across the whole tree of life: most taxa have now reliable phylogenies that allow addressing clear questions to, for example, disentangle effects of interspecific relationships, including for birds (Stoddard et al. 2017), mammals (DeCasien et al. 2017), and spiders (Hopfe et al. 2024). For rotifers, early phylogenetic studies did not manage to provide unambiguous relationships (Melone et al. 1998; Sørensen and Giribet, 2006), and the current use of phylogenomics did not improve the situation (Vasilikopoulos et al. 2024; Herlyn et al. 2025). Without a reliable phylogeny, most of the relevant questions highlighted by the panel of experts cannot be addressed, given the potential confounding factor of evolutionary relationships in cross-taxa comparative analyses (Garamszegi 2014). Without improving conceptual and comparative phylogenetic frameworks, many key evolutionary questions such as those addressing the genetic and ecological mechanisms underlying bdelloid speciation and adaptation (Q32, Q36, Box 3), the evolutionary role of homologous and horizontal gene transfer in asexual lineages (Q068, Q78), and the phylogenetic structure and morphological innovation within the group (Q96), remain unresolved. Notwithstanding such limitation, there is a broad interest in eco-evolutionary aspects of rotifers, especially related to their peculiar reproductive biology, the ability to survive desiccation, and the high level of horizontal gene transfer.

Box 3. Selected questions in the Evolution and Ecology theme after Phase 2. Ranked questions resulting from Phase 3 are highlighted in red.
Q32. What are the main ecological or genetic mechanisms driving speciation in bdelloid rotifers? (Gold)

Q36. Which genetic mechanisms enable rotifers to adapt to changes in salinity? (Gold)
Q78. Why are horizontally transferred genes so common and successfully integrated in bdelloid rotifers? (Silver)
Q96. What parasites and epibionts infect rotifers, how specific are these associations, and what are their impacts on host fitness? (Bronze)
Q68. In the absence of sexual recombination, how do alternative forms of homologous recombination contribute to adaptation in bdelloids?
Q79. How can phylogenetic relationships among rotifers inform our understanding of organ system evolution and morphological innovation?
Q80. What is the current phylogenetic structure of rotifers, and which clades are most closely related?

Human Impacts and Global Change

Anthropogenic pressures such as habitat degradation, biological invasions, and contamination are among the most severe threats to biodiversity. Disturbance often reduces native populations, disrupts trophic links, and accelerates community-wide biodiversity loss (Molinero et al. 2006). Yet much less is known about the effects of urbanization on microscopic animals, which may respond in different and sometimes unpredictable ways (Macêdo et al. 2020; Partemi et al. 2024; Han et al. 2025). These impacts generate many important questions (Q38–Q42). Anthropogenic pressures have created novel selective forces favoring pollution-tolerant rotifer species while destabilizing population dynamics, highlighting the need for robust quantification of the strength and direction of these responses. Key questions include how multiple stressors jointly affect rotifer development and reproduction (Q61, Box 4), how sensitive rotifers are to environmental and contaminant gradients across regions (Q04), and how such responses link to functional traits. An underappreciated dimension of global change is the biological invasion of microorganisms, with potentially far-reaching consequences (Macêdo et al. 2022; Oesterwind et al. 2025). Current understanding remains limited, risking misinterpretation and misuse of jargon in the field (Oliveira et al. 2019; Arcifa et al. 2020), with broad hypothesis testing still uncommon (Branco et al. 2023). This raises further questions about how rotifer abundance and diversity shift after invasions and the mechanisms underlying these changes (Q100). Meanwhile, wastewater treatment systems reveal the dual roles of rotifers as bioindicators and as active agents in remediation (Pajdak-Stós et al. 2023; Soto et al. 2019), prompting questions about the effectiveness of rotifer-based indices in detecting subtle shifts in water quality (Q16) and their contributions to nutrient recycling and microbial regulation in reuse-oriented systems (Q84).

Box 4. Selected questions in the Human Impacts and Global Change theme after Phase 2. Ranked questions resulting from Phase 3 are highlighted in grey.
Q84. How do rotifer communities influence nutrient recycling and microbial regulation in reuse-oriented systems like wastewater or agricultural reservoirs? (Gold) **Q100.** How does rotifer abundance and diversity change in response to invasive species introductions, and what mechanisms underlie these responses? (Gold) **Q61.** How do multiple environmental stressors affect rotifer development, survival, and reproduction across taxa? (Silver)

Q04. How sensitive are bdelloid rotifers to environmental stressors (salinity, temperature) and contaminants (e.g., metals, emerging contaminants), and are there locality-specific differences (e.g., Antarctic vs. Tropical)?

Q16. How effective are rotifer-based bioindicator indices in detecting subtle shifts in water quality across gradients of anthropogenic impact?

Methods

Both accurate identification and delimitation of species is crucially important to rotifer research. In the past, most researchers received taxonomic instruction from other researchers. Unfortunately, the number of rotifer taxonomists is declining, thus the opportunity for students to receive adequate training is nearly non-existent (Wallace et al. 2024). To build capacity and competency among new researchers, rotiferologists need to explore novel and efficient approaches (Q42, Box 5). A related issue is the need of assessing many samples rapidly and accurately. Artificial Intelligence tools may have the capacity to do this, but we need to have the procedures developed and tested and then made available at reasonable cost (Wallace et al. 2024) (Q46). Associated with accurate identification of species is the use of molecular data in two areas: (1) Appropriate genetic markers are needed to advance identification of species, so that changes in community structure can be monitored (eDNA) (Papakostas et al. 2016; Fröbius and Funch 2017) (Q35). (2) Genetic and morphological data should be integrated to advance phylogenetic studies. Ideally, a suite of genes including highly conserved, variable, and trait-specific genes for genetic studies is needed (Wilke et al. 2020) (Q34).

Box 5. Selected questions in the Methods theme after Phase 2. Ranked questions resulting from Phase 3 are highlighted in yellow.

Q34. What steps are required to link molecular sequence data with morphological traits in rotifers? (Gold)

Q46. Which tools or AI-assisted programs can support accurate rotifer species identification, and how can their efficiency be evaluated? (Gold)

Q35. What are the appropriate genetic markers for using eDNA in rotifer detection and monitoring? (Silver)

Q42. What educational tools are most effective for training new researchers in rotifer taxonomy and identification? (Bronze)

Q27. How do species within rotifer species complexes differ genetically and ecologically, and how can integrative methods (e.g., sequencing and habitat-based experiments) clarify their species status?

Q33. How can eDNA tools be used to detect cryptic rotifer diversity?

Q37. What empirical or modeling approaches best estimate rotifer dispersal rates across freshwater habitats, and how do estimates vary by environment type?

Q40. What are the key barriers to accurate rotifer species identification in potentially high diverse regions (e.g., Australia and the Neotropics), and how can they be overcome through targeted training or integrative methods?

Q45. What strategies can address the shortage of trained rotifer taxonomists in tropical regions, and how can this gap be sustainably filled?

Q44. Which PCR primers are most effective for amplifying rotifer DNA, and how do they perform across clades?

Q60. What are the molecular and physiological mechanisms underlying transgenerational plasticity in sexual reproduction in monogonont rotifers?

Q69. How can integrative approaches combining taxonomy, molecular tools, and ecological data accelerate rotifer biodiversity research?

Q72. How can a digital platform be built to compile and update historical literature on rotifers, including rare publications?

Q76. What are the genomic consequences of DNA damage and repair during anhydrobiosis in bdelloid rotifers?

Q88. How can standardized quantification of rotifer functional traits improve detection of anthropogenic impacts in small water bodies?

Q95. What mechanisms maintain the stable coexistence of multiple rotifer species in shared aquatic environments?

Populations

Using rotifer population dynamics to infer abiotic and biological drivers remains a core task in ecological and evolutionary research (Gilbert 1988; Lemmem et al. 2022; Réveillon and Becks, 2024). Recent studies show that rapid evolution and phenotypic plasticity can feed back on population dynamics even across short environmental gradients (Tarazona et al. 2019; Ramos-Rodríguez et al. 2020). This is especially clear in traits tied to persistence (e.g., resting egg banks, parthenogenesis, and dormancy timing), where small changes in hydroperiod or temperature can alter cohort structure and long-term resilience. How environmental variability (e.g., hydroperiod fluctuations) affects the evolution of diapause traits in rotifers, and how this influences long-term population persistence, remains a key question (Q53, Box 6). While correlative studies have documented associations between environmental variables and community composition, mechanistic understanding of such causal pathways driving population responses remains limited. For instance, understanding why bdelloid rotifers give birth to live young, and what adaptive advantages this confers in Antarctic environments (Q07), highlights how life-history traits may be causally linked to environmental constraints. This shift toward causal frameworks is critical because our ability to predict how rotifer assemblages will respond to global warming based on their diversity and distribution (Q24) will determine our capacity to forecast ecosystem functioning under novel conditions. Likewise, assessing whether geographically separated populations of the same rotifer species exhibit niche conservatism across environmental gradients (Q09) is essential for predicting their responses to environmental change. Such understanding is also important to evaluate the impact of invasive species on different local communities (Haubrock et al. 2024). Numerous studies on rotifers over almost a century have generated sufficient knowledge to apply them in fields such as aquaculture and ecotoxicology. However, basic research on their demography remains restricted to a few taxa such as *Brachionus calyciflorus* Pallas, 1776, *B plicatilis* Müller, 1786, *B. rubens* Ehrenberg, 1838, and *Plationus patulus* Müller, 1786 (Lemmem et al. 2022; Réveillon and Becks 2024). As a consequence, these species have become indispensable as food for larval stages in aquaculture or as bioassay organisms, while the potential of many other rotifer taxa, especially littoral species, has largely been neglected (e.g., in bioassays of toxicants that tend to sink to the bottom of ponds, lakes, and reservoirs).

Box 6. Selected questions in the Populations theme after Phase 2. Ranked questions resulting from Phase 3 are highlighted in blue.

Q53. How does environmental variability (e.g. hydroperiod fluctuations) affect the evolution of diapause traits in rotifers, and how does this influence long-term population persistence? (Gold)

Q09. Do geographically separated populations of the same rotifer species exhibit niche conservatism across environmental gradients? (Silver)

Q24. How well can rotifer community responses to global warming be predicted from diversity and distribution? (Silver)

Q07. Why do bdelloid rotifers give birth to live young, and what adaptive advantages does this confer in Antarctic environments? (Bronze)

Q10. What is the influence of climate change and new environmental conditions on the current distribution and abundance of rotifer species?

Q11. How have historical and evolutionary processes shaped the geographic distribution patterns of rotifers?

Q28. What is the extent and distribution of cryptic speciation across the rotifer phylum, and how can it be quantified?

Q38. How does dispersal of rotifers among connected freshwater habitats influence nutrient cycling and productivity in metacommunity frameworks?

Q52. Is population differentiation in rotifers driven more by isolation by distance or by environmental differences?

Q59. Which environmental cues or stressors trigger male production in rotifers, and how do these vary across habitats and taxa?

4. Discussion

4.1 Knowledge gaps and a hypothesis-driven future

Despite substantial advances in rotifer research, key gaps persist. The lack of reliable phylogenies is a major limitation for addressing evolutionary questions. Moreover, translating high-priority questions into testable hypotheses requires further effort. Persistent divergences in consensus, especially in *Evolution and Ecology*, suggest the need for more diverse approaches. These findings, together with the wide yet uneven international engagement observed in our research network (Fig. 2), reflect demographic biases common across ecological research (Tydecks et al. 2018). They also reveal that perceived research priorities vary across thematic areas, with stronger agreement in areas such as *Human Impacts and Global Change* and *Methods*, and more divergent views in *Ecosystems and Functioning.* Addressing these limitations will strengthen rotifer research and align the field with global scientific challenges.

One of the most significant challenges in rotifer research pertains to fundamental questions about their diversity and distribution. For instance, the persistently low number of rotifer species recorded in Latin America raises questions about underlying factors such as sampling bias and taxonomic effort (Fontaneto et al. 2012). This points to a broader issue of understanding global rotifer biodiversity, especially when considering the pervasive nature of cryptic diversity within rotifers and the potential for many species to remain undiscovered globally. The findings of this study contribute to illuminating these gaps, and planning scenarios for continuous taxonomic effort in multiple geographic regions, habitats and microhabitats. Implementing modern techniques will be essential for achieving a more accurate assessment of the full biodiversity of Rotifera.

A core objective of this initiative is to move beyond descriptive research towards a more hypothesis-driven future. By clearly articulating fundamental questions, this study lays the groundwork for generating testable hypotheses. For instance, questions about the mechanisms driving speciation in bdelloid rotifers (Q32/Gold) or the genetic basis of adaptation to salinity changes (Q36/Gold) directly invite the formulation and empirical testing of specific hypotheses. Similarly, e investigations how multiple environmental stressors affect rotifer development (Q61/Silver) and community structure (Q18/Gold) necessitate the development of predictive hypotheses that can be empirically evaluated. This shift is crucial for advancing rotifer biology from a phase of observation and description to one of mechanistic understanding and predictive power. Rotifers have the potential to serve as bioindicators of ecosystem health and can actively improve water quality. Recent studies (Davis et al. 2015; Pajdak-Stós et al. 2020) have demonstrated that certain rotifer taxa can significantly influence the composition of algal communities, including the suppression of harmful cyanobacterial blooms and toxin-producing species such as *Prymnesium parvum*. Their rapid reproduction, extensive grazing and tolerance of changing environmental conditions make them promising organisms for biomanipulation and ecological restoration strategies. This shift redefines rotifer research, expanding its focus beyond traditional monitoring and bioindicator roles to include the protection of aquatic ecosystem functioning and resilience.

Future plans to address priority questions in Rotifera research involve a strategic plan focusing on collaboration, funding, and dissemination, particularly between triennial IRS. Key initiatives include forming thematic working groups to translate questions into research, seeking targeted funding for collaborative proposals and taxonomist training, and promoting collaborations through webinars, virtual meetings, inter-symposium workshops, a digital collaboration platform, and researcher exchange programs. These efforts aim to advance research cohesively, address fundamental questions effectively, and enhance rotifers' relevance in ecology and conservation.

4.2 Philosophical dimensions

Our analysis of the 67 expert-selected research questions on rotifers (>50% consensus) showed that several had a philosophical dimension, touching on ontology, epistemology, and axiology (ethical and value-related questions), as outlined by Heger et al. (2024) who demonstrated the relevance of philosophical approaches to ecology. Ontological concerns, which deal with what exists and how it is connected, can be seen in Q22 (“Does global rotifer biodiversity reflect the biodiversity of species commonly used as indicators of environmental degradation?”) and in the more fundamental issue of “What constitutes a rotifer community?” implied in Q26 and Q97. Rotifer studies often rely on community definitions and biodiversity indicators that lack standardization or are based on subjective criteria and challenging definitions (Sládeček 1983; Fontaneto et al. 2007).

Epistemological challenges, concerning what we know and how we know it, are raised by Q25 (“To what extent does the structure of rotifer communities reflect short-term versus long-term climate-induced changes?"), which points to problems of temporal scale, and by Q78 ("Why are horizontally transferred genes so common and successfully integrated in bdelloid rotifers?"), which highlights issues of explanatory adequacy and levels of explanation. Other epistemological questions address methodological and conceptual issues. Q34 ("What steps are required to link molecular sequence data with morphological traits in rotifers?") exemplifies methodological pluralism by demanding the integration of molecular evidence, which captures processes at the level of genes and sequences, with morphological traits that emerge at higher levels of biological organisation. Q46 ("Which tools or AI-assisted programs can support accurate rotifer species identification?") and Q71 ("What strategies can sustain intergenerational transfer of taxonomic expertise in rotifer research?") highlight a growing tension between tacit, experience-based expertise and emerging technological approaches. In this sense, both questions reflect deeper epistemological debates about how scientific communities adapt to technological change and how knowledge persists (or is lost) across generations. These questions extend beyond improving identification: they ask whether taxonomic expertise can be formalised and delegated to algorithms or whether it remains embodied knowledge requiring mentorship.

At the same time, these questions also carry axiological and ethical implications, as an increasing reliance on AI reshapes whose expertise is valued, how authority is distributed, and what levels of transparency are acceptable in ecological practice. AI may expand data-processing capacity but also alters standards of evidence, accountability, and trust (Macêdo et al. 2023; Lu et al. 2025). Ethical and value-related aspects are also evident, for example, in Q100 ("How does rotifer abundance and diversity change in response to invasive species introductions?"), where the use of the term "invasive" rather than "non-native" has conservation and management implications (see

Soto et al. 2024). For further examples illustrating how ecological research engages with ontological, epistemological, and axiological issues, see Table 1 in Heger et al. (2025).

4.3 Model organisms

The 67 high-priority questions did not usually target individual species, but rather broader taxonomic groups. Only *Brachionus* Pallas, 1776, specifically *B. calyciflorus,* appeared in two questions focused on how environmental changes affect the expression of plastic traits within species complexes (Q50 and Q74). This reflects a critical research direction that leverages well-studied, phenotypically flexible species to understand adaptation in the face of climate change and environmental variability. Additionally, there are no high-priority questions specific to acanthocephalans or seisonids. Bdelloidea (mentioned eight times) and Monogononta (three times) also highlight pervasive interest in these groups. Interest in bdelloid rotifers stems from their extreme physiological tolerance, and the need to explain unique traits such as DNA repair during anhydrobiosis, the high incidence of horizontal gene transfer, and their responses to environmental stress and contaminants. Questions on monogonont rotifers, by contrast, often focused on resolving cryptic diversity and speciation, and on the molecular and physiological bases of transgenerational plasticity in sexual reproduction. Broader groups within Rotifera (see Sørensen and Giribet, 2006), including acanthocephalans and seisonids, were not specifically mentioned in any of the priority questions. Although bdelloid rotifers were specifically mentioned more frequently, many of the questions were only relevant to monogonont rotifers and not the broader phylum (e.g. resting eggs - Q55, Q56, Q57; male production - Q59; diapause - Q53, Q66).

4.4. De-colonizing Rotifera research

Reliance on expert elicitation can introduce geographical and disciplinary biases, particularly when certain regions or research traditions are over-represented. To amplify voices from historically underrepresented regions will strengthen the field and foster a more just, inclusive, and globally relevant vision for Rotifera research.

As in other research areas where persistent global inequities have been documented (e.g. Tydecks et al. 2018; Jian et al. 2025), our Delphi process also reflected imbalances. Experts working in freshwater ecosystems and researchers based in Europe and North America constituted the majority of contributors. Notably, no experts from the African continent participated, potentially reinforcing pre-existing geographic blind spots. At the same time, the workshop for Phase 3 of the Delphi process was held during the 17th IRS in Rio de Janeiro, the first time the IRS was hosted in the southern hemisphere. This location enabled unprecedented participation from researchers in South America, Central America, and the Caribbean, reducing common barriers to attendance and promoting greater regional representation in the consensus process. Such expanded participation is important because some research priorities—especially those concerning under-studied regions—may be overlooked when shaped predominantly by Global North perspectives. For instance, questions on rotifer diversity in Latin America (e.g., Q08), where sampling bias and limited taxonomic capacity strongly influence recorded species richness (López et al. 2025), might not surface as priorities without meaningful regional insight. By encouraging readers to examine the full list of 100 questions (Table S1), we aim to draw attention to topics that may have received fewer votes not because of low relevance, but because of limitations in participation, framing, or disciplinary familiarity.

Our study also revealed a gender imbalance among contributors in both Phases 1 and 2, with men representing a higher proportion of respondents (16% difference; Fig. 2), whereas gender parity was achieved during the in-person deliberation of Phase 3. The male bias in early phases likely reflects the predominantly male demographics at the education and career stages represented. This pattern mirrors well-documented structural inequities in academia, where women –although dominating student levels– remain underrepresented in senior and decision-making positions (Ceci et al. 2014; Wellenreuther and Otto 2016; Débarre et al. 2018; Salerno et al. 2019). Achieving parity in Phase 3 contrasted with typical trends and may relate to the fact that the 17th IRS was organized by a woman, as gender of organizers and senior authors strongly predicts the gender balance among participants (Débarre et al. 2018; Salerno et al. 2019). Such disparities are not only a matter of fairness; the composition of contributors can shape research agendas and determine whose perspectives influence scientific interpretation (Brizga et al. 2025; Débarre et al. 2018; Salerno et al. 2019). While the potential influence of the observed male bias on Rotifera research priorities was not assessed here, it may be an interesting consideration for future studies. More broadly, identity-based exclusion—including gender, LGBTQIA+ identity, or socio-economic constraints—can reduce both creativity and productivity in science, and is exacerbated by barriers such as event affordability and accessibility (Tulloch 2020). Although our questionnaire captured only three gender options, acknowledging these biases and monitoring representation helps support more inclusive and diverse research agendas. We recognize that equity is only one dimension of diversity, but addressing it is a meaningful step toward broader inclusion in scientific knowledge production (Débarre et al. 2018; Tulloch 2020).

4.5. Conservation efforts

Small waterbodies and shallow lakes often carry high species richness (Smolak and Walsh 2022), yet many of them are highly vulnerable to human impacts. Some of them are left to dry and converted for agriculture, real-estate development, or other human land uses. Larger waterbodies are not exempt from such pressures, and many waterbodies host endemic species and type localities (Kuczyńska-Kippen et al. 2025). Their loss results in a critical loss of biodiversity − in many cases biodiversity that was gone before it could have been detected and described. Such cases also cause serious gaps in our understanding of natural taxonomic diversity. Poorly studied systems, for example in South America, exacerbate this knowledge deficit in rotifer diversity (Q08), and reliance on extraterritorial material may also lead to underestimation of the true species richness within a given waterbody (Q42).

Conservation efforts targeting waterbodies with high species richness, high endemic taxa, or type localities should involve public engagement and local governmental support (Q87). Rotifer research is often hidden in limnological studies, meaning that national and international conferences may carry valuable information on unique freshwater habitats that may go unnoticed. Therefore, systematically compiling available data from such sources could substantially enhance our understanding of rotifer diversity and support the preservation of waterbodies that harbour exceptional biological value.

Conclusion

By leveraging a comprehensive Delphi process, our study has synthesized the collective expertise of the international rotifer community to identify and prioritize fundamental questions guiding rotifer research. While not intended as an exhaustive list, it represents a collective snapshot of the field's current priorities and emerging directions, offering a curated roadmap for students, researchers, funding agencies, and environmental policy. Our aim was to stimulate new collaborations and interdisciplinary research, fostering the formulation and testing of new hypotheses, and promoting integration of rotifer studies into broader ecological and evolutionary contexts.

Author Contributions

Conceptualization: Rafael L. Macêdo, Gissell Lacerot and Jonathan M. Jeschke. Investigation, methodology, validation: Rafael L. Macêdo, Gissell Lacerot and Jonathan M. Jeschke. Data curation: Rafael L. Macêdo and Gissell Lacerot. Formal analysis: Rafael L. Macêdo. Writing—original draft: Rafael L. Macêdo. Visualization: Rafael L. Macêdo, Gissell Lacerot, Carlota Solano-Udina, Melanie D. Borup, Marco Antonio Jiménez-Santos, Diego Fontaneto. Writing—review and editing: All authors wrote sections of the Results and Discussion and critically reviewed and edited the entire manuscript.

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Conflict of Interest

None declared.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

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FIGURES

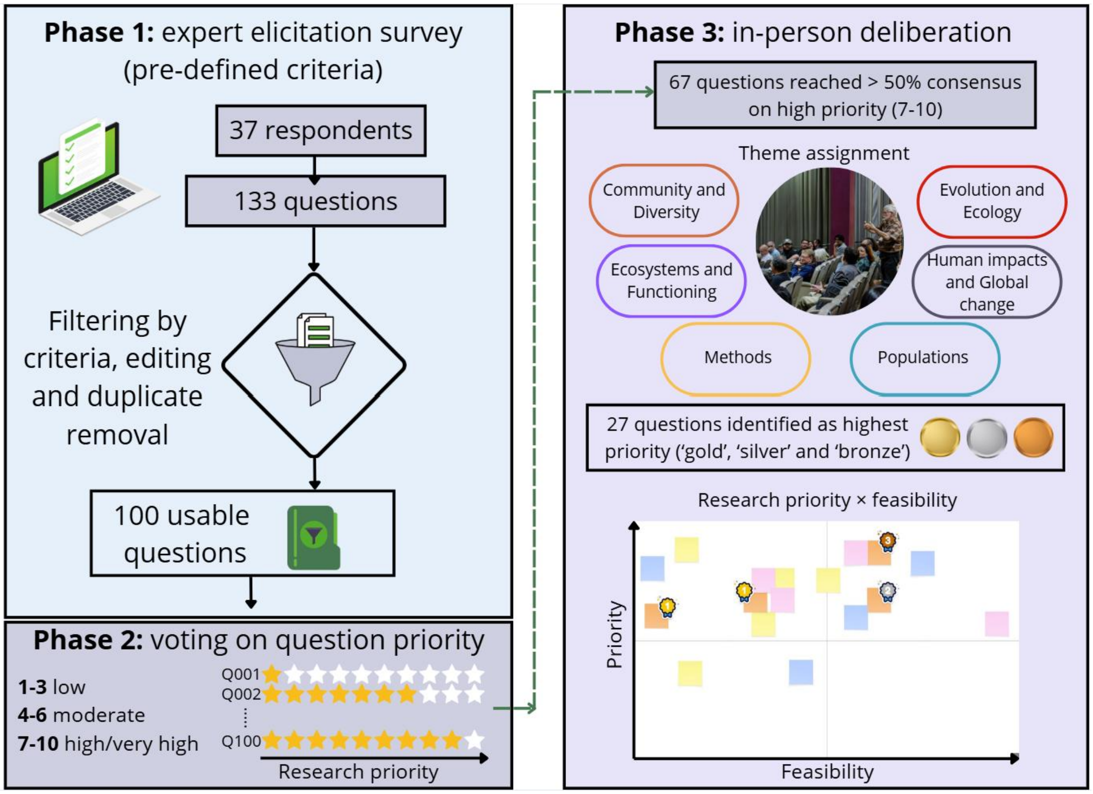

Figure 1. Workflow of our approach to identify research priorities in Rotifera research. Phase 1 involved expert elicitation and filtering of 133 proposed questions into 100 usable ones (see Supplementary Document S1). Phase 2 focused on expert scoring to prioritize the questions, and Phase 3 involved in-person deliberation, theme assignment, and identification of the top 27 high-priority questions.

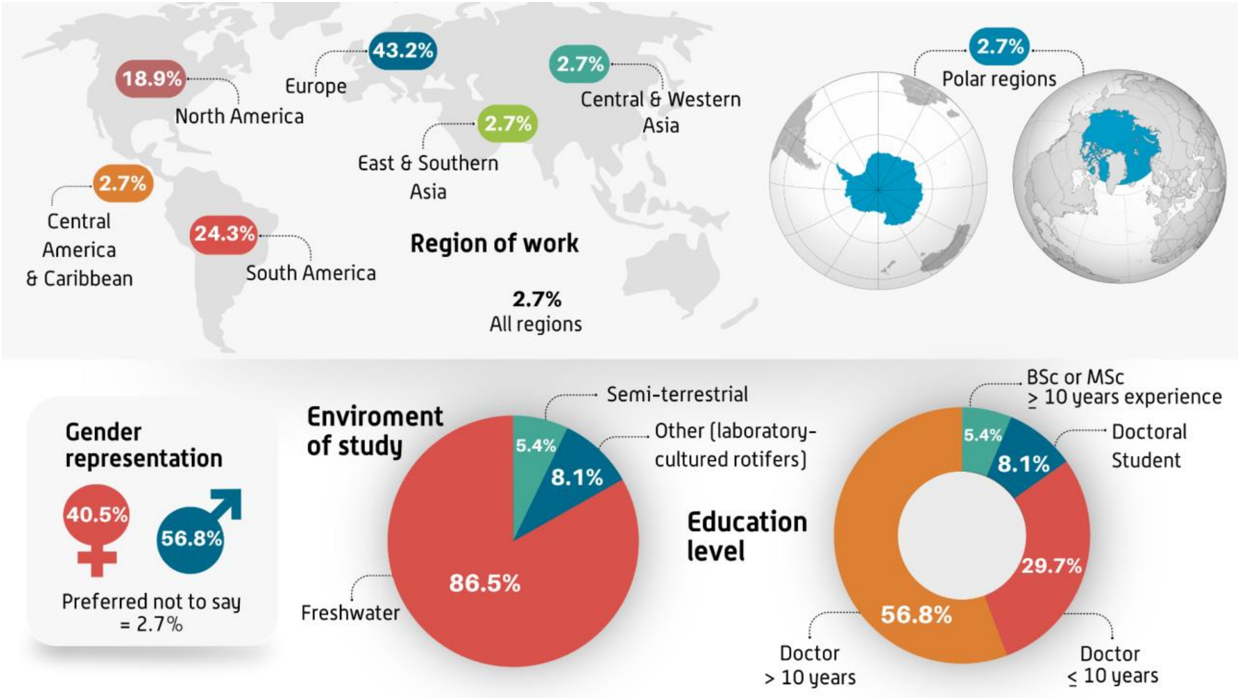

Figure 2. Demographic composition of the expert panel (n = 37) during Phase 1. The map shows the geographic distribution of experts by region of work, expressed as percentages of total participants. Accompanying charts present the academic backgrounds, education levels, ethnic groups, and gender representation.

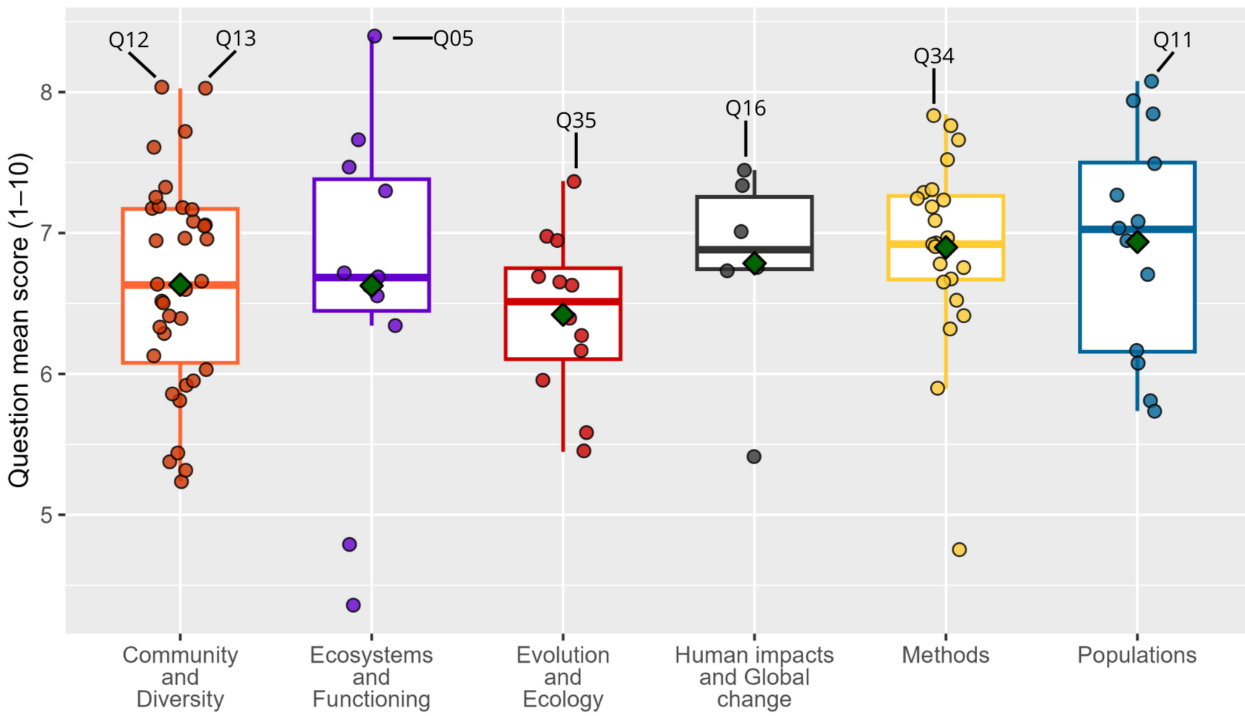

Figure 3. Distribution of the 100 questions mean scores by research theme. Each point represents the mean expert score for one question during Phase 2, boxplots indicate the interquartile range, whiskers represent the maximum values within 1.5 interquartile ranges, horizontal lines within each box show the median, and diamonds mark the mean values. This visualization highlights variation in perceived priority across themes. Scores did not differ significantly among themes (Welch's ANOVA, $p = 0.35$)

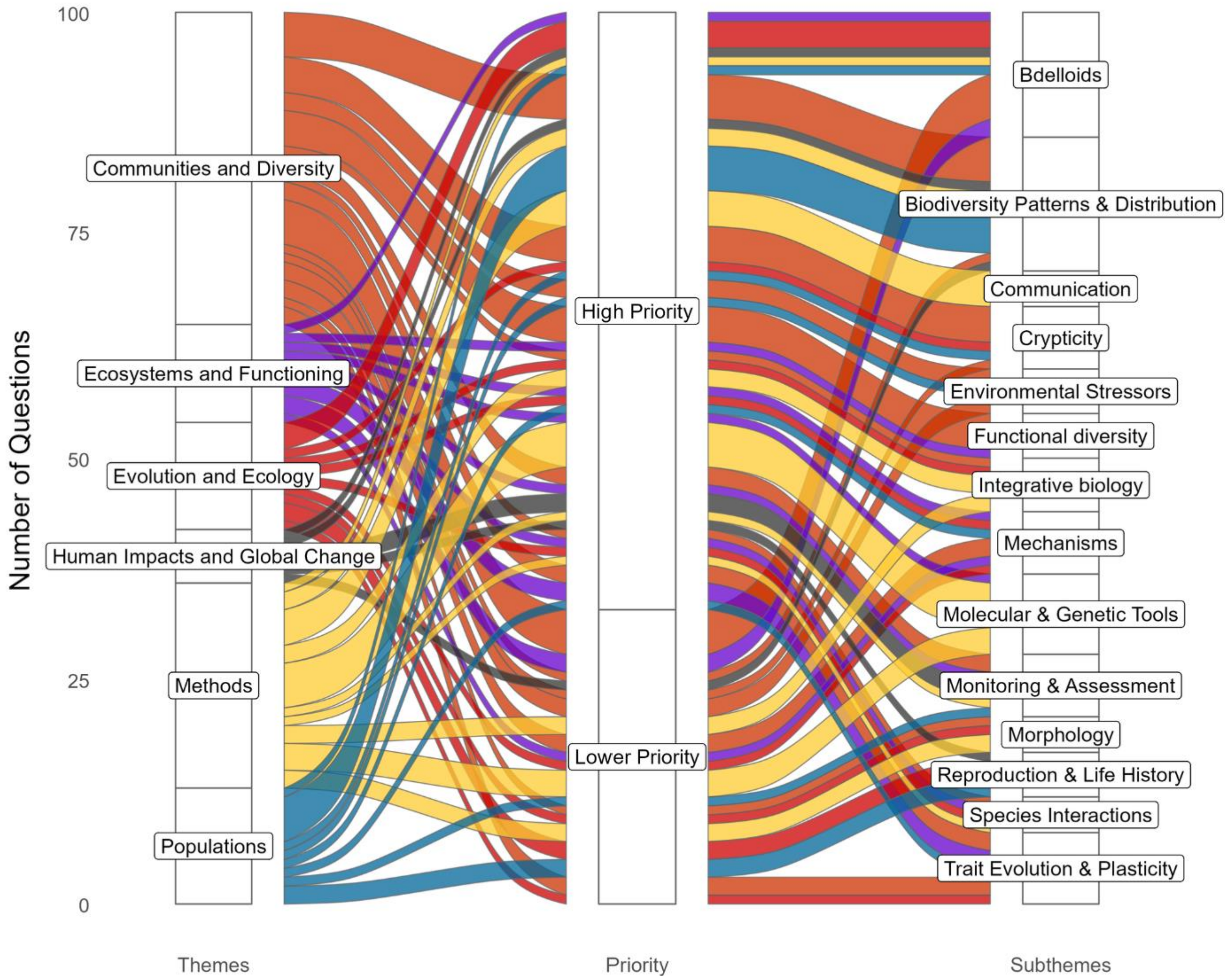

Figure 4. Sankey diagrams showing flows from Themes → Priority classification → Subthemes from Phase 1 to 3. Widths reflect the number of questions considered during Phase 3, highlighting absolute research focus. Subthemes were grouped based on shared conceptual keywords.

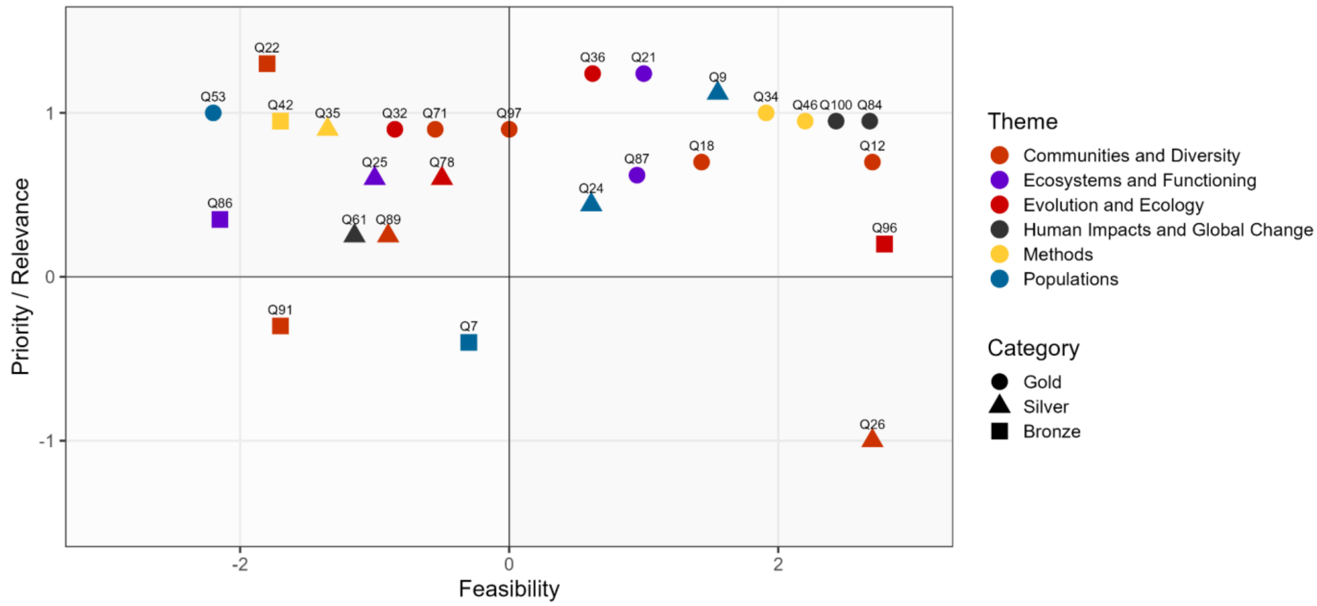

Figure 5. Phase 3 results: highest-priority questions placed on a two-axis grid (priority × feasibility), enabling experts to visually compare and negotiate the relative importance and practicality of candidate research questions. The original panel resulting from the in-person exercise during the workshop is available at Genially.com for close inspection.