

# Systematics as a cornerstone of parasitology: overview and preface

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## OVERVIEW AND PREFACE

Systematics is a rich scientific discipline by which the components of biodiversity are described, named and enumerated, and by which their relationships are described. To paraphrase a colleague, the field encompasses three simple questions: *what is it, where does it come from* and *how is it related?* Individually, and together, these questions may be applied to any biological entity. In the realm of systematics they are covered in turn by taxonomy, biogeography and phylogenetics. Historically and most commonly, the element chosen as the focus for these studies is the species. Evolutionary processes act on all heritable components of life that undergo natural selection. Thus, these three questions are just as pertinent when working with genes, their products, or other molecular units (e.g. microRNAs). By addressing these questions in light of evolution, systematics offers a framework to view life in space and time, and provides an ever-expanding tool-set to test hypotheses that explain patterns and reveal underlying processes. Parasitologists necessarily consider their organisms in light of their interactions with hosts (including vectors, intermediate, reservoir, paratenic and final hosts); this differentiates their work from that of many other biologists as they need to view animals holistically, as integral parts of their environment interacting with other organisms. Since few organisms escape parasitism (Poulin, 2011a), the nature of these intimate interactions is worthy of considerable attention in natural history, not just by parasitologists. Meanwhile, for parasitologists, the opportunities gained from viewing both hosts and parasites in the light of systematics, provides deeper insights into the nature of parasitism.

Parasitologists have been at the forefront of addressing the questions *what is it* and *where does it come from* (or *who in whom?* as phrased by Combes, 2001), because answers to these questions are the basis for diversity assessment, and of course parasite and vector control. Many multi-million dollar

investments are founded on detecting and targeting the right species of parasite or vector, over various geographic scales, in a bid to control disease. The importance of understanding *how is it related* may not always have been so obvious, or tractable. Indeed, if a closely related parasite or host species is not a problem, why should we bother understand it further? From a wider perspective, how does knowing a parasite or host's evolutionary history inform us? The answers only become obvious when we find we have missed a reservoir host, or developed a control programme or applied a therapy based on a model system so vastly different from the parasite we wish to control that it is worthless. When we can harness its powers to reveal, explain and predict biological patterns fully, systematics becomes an all-important theme in parasitology, and not just a starting point.

The full influence of an evolutionary approach, underpinned by systematics, has only become apparent and tractable to most parasitologists through the pursuit of genes and genomes. Molecular biology has provided additional (or independent) characters for understanding species and speciation, interrelationships and phylogenetics, as well as a biochemical basis for understanding the nature of development, phenotype and the remarkable abilities of parasites to be not only unnoticed or unaffected by host defences, but to manipulate the biology of their hosts. To gain complete insight from a comparative biological approach, the lens of systematics facilitates focus and clarity, regardless of biological system or scale (taxonomic, temporal or spatial). Arguably, systematics is **the** cornerstone of parasitology.

In this Special Issue of *Parasitology* contributors working on a diversity of parasites (distributed over very different taxonomic and geographic scales) demonstrate the pursuit of the three principal questions underlying systematics, as applied to a variety of topics and challenges in parasitology. One of the single most unifying goals of systematics is to assemble the Tree of Life: a phylogeny that links all organisms back to their common ancestors and origins, which in turn provides a framework for taxonomic organisation and classification, as well as tools to test hypotheses. Established on the principles of homology and shared ancestry, phylogenies may

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be estimated from morphological characters, genes, proteins, or any other unit that is heritable. Some argue that the tree metaphor is outdated, no longer accounting for the multiple instances of horizontal gene transfer, particularly widespread amongst unicellular lineages, including parasites (Schaack *et al.* 2010). However, whether a tree or a network, the efficacy of any method used to recapture historical patterns of divergence events accurately relies upon the use of homologous characters, selection of models of evolution appropriate to the data being used, sufficient sampling of organisms and characters, and methods by which results can be assessed, compared and tested. There is always room for error and disagreement, but working towards a resolved tree, or branch of it, remains a valuable goal, from which we can move ahead.

Undoubtedly, for systematic microbiologists the molecular revolution has been transformational and empowering, because it provides an entirely new view of interrelationships (especially at the base of the Tree of Life), reveals a hidden diversity of microscopic forms and arms them with tools for investigating parasite biology. Entire Domains of life, owe their recognition to molecular data, while other major branches are being significantly reorganized, as more data accumulate and more taxa are sampled. Walker *et al.* (2011) provide a comprehensive overview of branches of the eukaryote tree, which highlights the diversity of parasites and their multiple origins, the plethora of new names and the key morphological features that can still be used to recognise and differentiate phenotypes. The authors provide an excellent state-of-the-art primer on all eukaryotic groups as a downloadable supplementary document. With the advent of environmental sampling, along with the growth in metagenomics and new/next generation sequencing, the eukaryote tree will continue to recruit new parasites as they are found, as well as gain stability and resolution as more taxa and data are used to build it.

Morphology has been at the forefront of systematic parasitology since its origins, particularly for researchers concerned with multicellular animals. For many it is the first port of call for diagnosis, identification and assessing relatedness. Although the 'debate' pitching morphology versus molecules is no longer pursued (since both phenotype and genotype are recognised as integral components in understanding interrelationships and the nature of change through time), researchers are constantly being pulled in one direction or another over the spectrum that links genotype to phenotype. This comes as a result of fashions, and the emergence of new tools and techniques that seem to provide quicker means of delimiting and diagnosing species, or enumerating differences within species. However, if we short-change systematics by ignoring the evolutionary history of the whole organism when

looking for simple diagnostic tools to address the *what is it* question, we risk choosing inappropriate markers that fail to differentiate between species or perhaps worse, identifying species incorrectly (e.g. see Goldstein and DeSalle, 2011; Dasmahapatra *et al.* 2010). It is preferable to assemble the fullest picture from all available evidence, and the taxonomic and phylogenetic limits (i.e. accuracy) of any chosen diagnostic tool or marker need to be known before it is implemented, especially by non-specialists. Perkins *et al.* (2011) highlight, with examples, how morphology has deceived the systematic study of some parasite groups, most notably the small and microbial, and make the case for a continually updated integrative approach, particularly where morphology is scarce or difficult to work with. Caira (2011) draws upon the experiences of community-led integrated approaches to modern systematic cestodology, and highlights how techniques that take short-cuts (such as molecular taxonomy) may confuse and mislead efforts to reveal the complexities of parasite biodiversity. Short-cuts necessarily cut corners, but the importance of systematics is too great to allow its goals to be compromised or ignored. Some types of data are easier to collect than others but parasitologists, perhaps above all, cannot afford to lose sight of the importance of accurate identification. These authors highlight how integrative taxonomy is as much about integrating researchers as it is about integrating research results and approaches (see also Dayrat, 2005; Padial *et al.* 2010). Reciprocal illumination, where the results from each data set inform the interpretation of another, remains the preferred approach.

Molecules contribute through their enhanced ability to differentiate within and between species, and to reveal hidden diversity in the form of cryptic species. The sheer number of available discrete, measurable molecular differences within and between species has supported a wider appreciation of cryptic diversity, but once again any differences need to be enumerated and viewed in light of morphology and the whole organism (the species). Nadler and Pérez-Ponce de León (2011) provide an extensive review of the topic, and focus on helminths, which have been shown to demonstrate high levels of cryptic diversity (Poulin, 2011b). The authors reflect on species concepts in theory and in practice, and consider how important it is to resolve the underlying questions of systematics fully in achieving the wider goals of parasitologists.

Recognising what something is can be a daunting task in biology, and no less so for parasitologists who often deal with small, cryptic, fast-evolving organisms with complex life cycles, and which undergo massive morphological transformations during development. Little wonder that, in the absence of molecular tools, completing a parasite's life cycle,

determining its ontogeny, mode of transmission and host use, was more than enough to secure a well-earned doctorate in parasitology for much of the first half of the 20th century. Given the parasitologist's heightened sensitivity to species recognition and delimitation, not least in the face of molecular and cryptic diversity, vector systematics is no less important. Zarowiecki *et al.* (2011) tackle recent advances in species and speciation studies in the context of vector identification and control, with a particular emphasis on mosquito systematics. Species-rich, geographically widespread, and of significant economic and medical importance, mosquitoes offer considerable challenges to systematic parasitologists. Without tackling these challenges, efforts to control vectors may be ill conceived, costly and ineffective.

As a result of the intimate relationship between parasites and their hosts, parasites cannot be understood fully without understanding their dependence, interactions and evolutionary radiation with their hosts; e.g. see Combes (2001); Poulin (2007); Morand and Krasnov (2010). Miller *et al.* (2011) review a wealth of data, gleaned from long-term systematic surveys of teleosts and their trematodes from the Great Barrier Reef, in order to reveal meaningful patterns of host-specificity (a fundamental property of parasites; Poulin *et al.* 2011). The results are disturbing, with many host-parasite associations neither predictable nor explicable, despite the wealth of data and extensive sampling. The complexity of host-parasite associations and evolution is such that extrapolating patterns, even from numerous examples, may be difficult and certainly misleading. Fine-scale systematic sampling of biodiversity is still required to understand host-specificity and complex ecosystems fully, and to formulate reliable predictions from meaningful patterns. Nevertheless, the approach taken by Miller and colleagues helps differentiate the known from the unknown, and to frame tractable questions, which might be addressed by further sampling.

Encouragingly, among less diverse parasite systems, with simpler life cycles, the nature of the intimate relationship between hosts and parasites is more tractable, and may be untangled by experimental manipulation. Johnson *et al.* (2011) build upon extensive phylogenetic sampling of bird lice in revealing and testing patterns of parasite distributions in hosts, thereby addressing a fundamental concept in parasitology—namely, that parasites evolve towards increased host specialization over time. Although rare, louse dispersal from one host to another occurs frequently enough to maintain lice species as overall generalists across multiple host species. Myth-busting is an important part of science, and no less so in parasitology, which has accumulated its fair share of false 'laws', untested dogma, inaccurate observations, and inadequate

extrapolations (e.g. see Brooks and McLennan, 1993).

The systematics tool-kit is forever being improved upon and established hypotheses, along with myths, benefit from reanalysis when new data become available; the era of genomics and new generation sequencing has provided many such opportunities. One such example is provided by Silva *et al.* (2011) who tackle the question of how, and from where, malaria parasites arose in humans. An accurate and credible phylogeny, which charts the evolutionary radiation of the parasite, is at the heart of resolving these questions. However, in spite of the wealth of molecular data for *Plasmodium* (entire nuclear genomes for some species), phylogenetic estimates are not always readily available or widely accepted. Through careful analysis, Silva *et al.* (2011) provide not only a tree, but also an estimation of likely divergence times of major lineages. The results and interpretation contest the notion that apes are reservoir hosts for human malaria; indeed, the reverse seems to be more accurate. Furthermore, a comparative evolutionary approach further highlights the need to select appropriate model systems in an attempt to understand human parasites.

There are three prominent examples where the study of shared histories involves the consideration of nested trees. They include biogeography (reconciling distributions with historical patterns of diversification), the reconciliation of gene trees and species trees, and reconciling evolutionary patterns of host-parasite relationships influenced by cospeciation and coevolution. Detecting and explaining incongruence among these sets of nested trees, reveals phenomena such as vicariance events, gene coalescence, and host switching. Cophylogeny mapping is a popular and intuitive approach to untangling nested trees, although it may be computationally difficult to achieve (Page, 2003). In parasitology the method comes to the fore in reconciling host and parasite coevolutionary histories.

In the case of molecular phylogenetics cophylogeny mapping reveals incongruence but does not necessarily provide solutions in reconciling different gene trees when pursuing them as estimates of species trees. Knowles and Klimov (2011) apply novel Bayesian approaches in species-tree estimation, using multi-locus molecular data from bird mites, where individual gene trees are in conflict with one another. Conflict is especially common when rates of speciation are high, or speciation events relatively recent, as is likely in the system chosen by these authors. The new species-tree approach overcomes much of the conflict and exemplifies how understanding its origin amongst data sets provides sound biological insights and, importantly, possible remedies for improved sampling. McDonagh and Stevens (2011) show that much is to be gained from comparing and contrasting individual gene trees

with one another, with combined analyses and traditional taxonomies barely influenced (as yet) by molecular data. Such investigative approaches remain the starting point for many studies, as they provide a unifying, testable framework for denser sampling, species tree resolution, character mapping, and even the detection of species hybrids.

Environmental and climate changes are capable of profound effects on biodiversity and ecosystems. Increasingly, added pressures from urbanization, globalization and other human-mediated change are being shown to have consequences for biodiversity, while recent studies have begun to reveal the effects on parasites too. As regulators of host populations (Poulin, 2007), the role of parasites in changing environments is little studied but no less important (e.g. see Brooks and Hoberg, 2007). When introduced to non-native systems the effects of parasites are largely unpredictable. Biological invasions, whether caused directly or indirectly by humans, are expected to reach high population numbers if they arrive in areas with few predators, or without parasites (Torchin *et al.* 2003). If parasites are introduced with their hosts, there are opportunities for host-switching resulting in devastating effects on new host species. Verneau *et al.* (2011) show how parasite phylogenetics reveals recent interactions between helminth parasites of native and non-native (escaped or released captive) freshwater turtles. The rich monogenean fauna of turtles is used to demonstrate complex interactions and multiple instances of host-switching, and provides an important reminder as to the potential problems faced by conservation biologists in disregarding parasites when reintroducing hosts or moving them from one area to another. Host-switching is not uncommon and can lead to explosive radiations and the rapid colonisation of new hosts often extending the geographic ranges of parasites.

Monitoring change through time allows significant or unusual perturbations to be differentiated from normal variation, whether cyclical or seasonal. Often, it is only through sustained, long-term monitoring, that benefits become apparent. Parasites, especially those with complex life cycles, are excellent ecological and trophic indicators. Palm *et al.* (2011) take the example of monitoring the parasites of cultured finfish to demonstrate the effects of changes to feeding strategy (mariculture conditions) and/or environmental disturbance on parasite fauna (diversity and prevalence). Parasites may act as useful bioindicators of ecosystem health and change, but their efficacy begins with the ability to recognise them accurately. Once again, the need for taxonomic expertise, training and awareness highlights a bottleneck that requires parasitologists to support systematics (Brooks and Hoberg, 2001; Hoberg, 2002).

All too often in comparative studies, particularly in measurements of life-history traits, meaningful

understanding of variation is confounded by lack of independence of data arising from common ancestry. The application of phylogenetically independent contrast methods begins with systematics and relies on the fruit of 'Tree of Life' studies. Ponlet *et al.* (2011) test the consequences of animals hosting a diversity of parasites. Parasite diversity is expected to add to a host's investment in immunity. By measuring several morphological features of internal organs in rodents, as a proxy for investment in immunity (immunocompetence), and enumerating parasite diversity, the authors found that both male and female rats respond significantly (although differently) to indicators of parasite species richness and parasite diversity. Key to this study was the availability of phylogenetic trees for both hosts and parasites, for developing indices that could be analysed with evolutionary history independently accounted for, when linking cause and effect.

Frequently when taking a multi-faceted approach, the benefits of considering the whole far outweighs the sum of the individual parts. Siddall *et al.* (2011) take stock of some of the harvest from their Tree of Life studies on leeches and incorporate systematic studies on bacterial symbionts found in, and genes expressed by, the salivary glands of different leech species. The result is a rich and productive insight into leech biology, its relationship with its symbionts and the effect of this symbiosis on molecular function (s). Protein, symbiont and leech phylogenies provide trees within trees within trees, and raise the prospect that many previously intractable questions are within reach via the systematics tool kit.

Non-systematists often despair when names and classification systems change as a result of systematic revisions or the redrafting of taxonomic keys and schemes. They are not alone as systematists often despair too, especially when synonyms accumulate, confusion reigns and the user community lags behind in implementing the latest results. In spite of the flux that systematics can cause, its goals are stability, utility and an accurate account of interrelatedness to be used as the basis for classification and interpretation. Given the sheer number of species and the richness of biodiversity, systematists face a daunting task ahead. The continued loss of taxonomists and taxonomic expertise is also of concern (Poulin and Leung, 2010). For most systematists, the key to progress is to choose the taxa and sampling strategy likely to give a realistic and usable framework for validation and testing. For parasitologists, who generate and prioritise the questions and hypotheses, progress is contingent upon reliable taxonomy and phylogeny and the implementation of systematic tools. Regardless of which branch, twig or leaf of the Tree of Life one works with, and however narrow or focussed one's perspective in parasitology, systematics is a cornerstone that underpins the understanding of parasites and parasitism.

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