



Modern Strategies for Diagnosis and Treatment of Parasitic Diseases

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Parasites are very widely distributed in the environment and form complex relationships with their hosts, forming host–parasite systems. They have various life strategies, either parasitizing on the body of the host (ectoparasites) or inside it (endoparasites). They can form both permanent, often long-term, and temporary relationships and can demonstrate varying degrees of host specificity, from specialists to generalists. They often have complicated, complex life cycles characterized by various host strategies, which are selected at different stages of development. Moreover, their structure and life functions can range from microscopic, single-celled protozoans and multicellular, relatively primitive helminths to parasitic arthropods, complex animals with a nervous system demonstrating a highly advanced structure and functions [1–4].

Our understanding of a particular group of parasites depends to a great degree on the nature of the group and its region. Undoubtedly, parasites known to be pathogenic towards humans, pets, and farm/breeding animals are of greater interest; however, relatively little is known about these parasites. They function in an environment that is continually changing as a result of anthropogenic pressure and climate change and is subject to the influence of a range of complex conditions, e.g., in a wide range of reservoir hosts and their expanding geographical ranges. They can also become resistant to previously used treatment methods. Studies also indicate that their diversity is most likely much greater than currently recognized, as new species continue to be discovered and described and the introduction of molecular testing has revealed the presence of so-called cryptic species, which are difficult or impossible to identify based on morphoanatomical methods [5–19].

Therefore, there is still a lack of effective methods for detecting many parasites and for diagnosing and treating parasitoses and their related symptoms, such as allergic reactions and pathogen transmission. There is also a need to improve and develop parasitological monitoring and forecasting methods in response to local and global environmental changes, such as climate change, which often expand the range of the host and thus the parasite [20–25].

It is hence essential to improve our research approaches with the support of modern techniques; one such useful, even indispensable, armamentarium for parasitological research, and the standard in some areas, comprises methods based on molecular biology. Such approaches are increasingly used in the taxonomic identification of parasites, especially microscopic organisms that are difficult to identify, or parasites with evolutionary modifications which can obfuscate their rank and systematic position. Such analyses are of great importance for the study of biodiversity, geographical spread, and pathogen transmission, and for determining relationships and analyzing dependencies in complex host–parasite systems. Molecular methods are an indispensable tool within the spectrum of advanced diagnostic methods (especially in the context of hard-to-detect parasites) and therapeutic methods (such as drug and vaccine design) [26–46]. As such, researchers must develop diverse, unique approaches to various research topics, depending on the group being studied, the scientific problem, the scope and area of research, and their specific goals.



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). The current Special Issue has therefore become not only a collection that shows the diverse nature of the research issues in contemporary parasitology, but also a platform for discussion on the possibilities of using various methods from the field of molecular biology [47–54]. The area has recently been subject to an enormous growth in research and, as such, its status requires constant updating and specialized verification; hence, review studies critically analyzing the current state of the knowledge in this area are also invaluable.

Molecular methods have revolutionized a number of parasitological specialisms, including the study of single-celled organisms, which were previously difficult to detect and identify due to their microscopic size and the small range of features they have that are useful in taxonomic studies based on morphoanatomy [47–53]. These methods can be used in a wide spectrum of research, such as population studies aiming to detect parasites and estimate the frequency of their occurrence in various human populations and communities, thus monitoring changes and detecting new threats [47]. One such study employed molecular methods to detect and identify Blastocystis sp. in Polish soldiers stationed in the Republic of Kosovo. Research has shown that a longer stay in a new environment can result in a significant increase in infection; these findings may further the development of prevention procedures [47]. In terms of environmental monitoring, research related to the detection of parasites in various reservoir hosts is also important. One such important, although poorly understood, parasitological group comprises bats, which may be a source of parasites with zoonotic potential [48]. Thus, in a study of three species of common and widely distributed bats, Toxoplasma gondii, Neospora caninum, and *Encephalitozoon* spp. microsporidia DNA was detected; this study offers new data in this field, not only on the European scale, but also on the global scale. It also confirms the role of bats as reservoir hosts of parasites important to humans and the animals associated with them [48].

Expanding and extending the range of molecular tools available to us is of great importance for parasitological diagnostics and may also change our therapeutic approach to parasites [49–52]. This may include, for example, new opportunities to use improved, more effective diagnostic tests for common and often dangerous intestinal parasites that cause disease and death, such as *Cryptosporidium* spp., *Giardia intestinalis*, and *Entamoeba histolytica* [49]. In turn, research conducted around the world, based on a new approach using a combination of genomics and proteomics, has significantly contributed to the development of new diagnostic tools, vaccines, and drugs for cryptosporidiosis [50]. Another modern approach that employs molecular research tools has also allowed for the development of innovative diagnostic methods for detecting human amoebiasis, especially the identification, genomic analysis, and examination of the genetic diversity of *Entamoeba* spp. and the association of specific genotypes with various forms of human amoebiasis [51].

The development of research methods and new therapeutic approaches is clearly visible in a study of the pathogenic protozoa *Trypanosoma* spp. [52,53]. This study's findings indicate that cyclophilin (CyP) enzymes can be used as early biomarkers of the effectiveness of a treatment for trypanosomiasis (Chagas disease), which is caused by *Trypanosoma cruzi* [52]. Similarly, the introduction of modern diagnostic methods has allowed for the timely and accurate diagnosis of human African trypanosomiasis caused by *T. brucei*. This early intervention is crucial for its effective treatment, and the introduction of new therapies may contribute to the complete elimination of this disease in the near future [53].

Modern methods based on integrative approaches that combine achievements and tools from various specialties have also fueled the development of scientific research on multicellular parasites; these approaches have generated improvements in diagnostic methods and led to the introduction of new therapies useful in medicine and veterinary medicine. An example is the study of parasitic nematodes in the gastrointestinal tract of small ruminants, which cause significant losses on animal farms. Research, and especially immunological research, is employed in the search for new strategies to prevent parasitosis and develop antiparasitic vaccines [54].

While the scope of the possibilities associated with the modern parasitological research methods presented herein will undoubtedly not exhaust the topic, they will hopefully serve as a source of inspiration for new ideas.

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References

- 1. Poulin, R.; Morand, S. The diversity of parasites. Q. Rev. Biol. 2000, 75, 277–293. [CrossRef] [PubMed]
- 2. Bush, A.O.; Fernández, C.; Esch, G.W.; Seed, J.R. *Parasitism: The Diversity and Ecology of Animal Parasites*; University Press: Cambridge, UK, 2002; p. passim.
- Park, A.W.; Farrell, M.J.; Schmidt, J.P.; Huang, S.; Dallas, T.A.; Pappalardo, P.; Drake, J.M.; Stephens, P.R.; Poulin, R.; Nunn, C.L.; et al. Characterizing the phylogenetic specialism–generalism spectrum of mammal parasites. *Proc. R. Soc. B* 2018, 285, 20172613. [CrossRef]
- 4. Runghen, R.; Poulin, R.; Monlleó-Borrull, C.; Llopis-Belenguer, C. Network analysis: Ten years shining light on host–parasite interactions. *Trends Parasitol.* **2021**, *37*, 445–455. [CrossRef] [PubMed]
- 5. Poulin, R. How many parasites species are there: Are we close to answer? *Int. J. Parasitol.* **1996**, *26*, 1127–1129. [CrossRef] [PubMed]
- Dobson, A.; Lafferty, K.D.; Kuris, A.M.; Hechinger, R.F.; Jetz, W. Homage to Linnaeus: How many parasites? How many hosts? Proc. Natl. Acad. Sci. USA 2008, 105, 1482–11489. [CrossRef]
- 7. Leung, T.L.F.; Keeney, D.B.; Poulin, R. Cryptic species complexes in manipulative echinostomatid trematodes: When two become six. *Parasitology* **2009**, *136*, 241–252. [CrossRef]
- 8. Nadler, S.A.; De León, G.P. Integrating molecular and morphological approaches for characterizing parasite cryptic species: Implications for parasitology. *Parasitology* **2011**, *138*, 1688–1709. [CrossRef]
- Poulin, R. Uneven distribution of cryptic diversity among higher taxa of parasitic worms. *Biol. Lett.* 2011, 7, 241–244. [CrossRef] [PubMed]
- 10. Ghai, R.R.; Chapman, C.A.; Omeja, P.A.; Davies, T.J.; Goldberg, T.L. Nodule worm infection in humans and wild primates in Uganda: Cryptic species in a newly identified region of human transmission. *PLoS Negl. Trop. Dis.* **2014**, *8*, e2641. [CrossRef]
- Bray, R.A.; Cribb, T.H. Are cryptic species a problem for parasitological biological tagging for stock identification of aquatic organisms? *Parasitology* 2015, 142, 125–133. [CrossRef]
- 12. Jephcott, T.G.; Sime-Ngando, T.; Gleason, F.H.; Macarthur, D.J. Host-parasite interactions in food webs: Diversity, stability, and coevolution. *Food Webs* **2016**, *6*, 1–8. [CrossRef]
- Nilsson, E.; Taubert, H.; Hellgren, O.; Huang, X.; Palinauskas, V.; Markovets, M.Y.; Valkiūnas, G.; Bensch, S. Multiple cryptic species of sympatric generalists within the avian blood parasite *Haemoproteus majoris*. J. Evol. Biol. 2016, 29, 1812–1826. [CrossRef] [PubMed]
- 14. Izdebska, J.N.; Rolbiecki, L. The biodiversity of demodecid mites (Acariformes: Prostigmata), specific parasites of mammals with a global checklist and a new finding for *Demodex sciurinus*. *Diversity* **2020**, *12*, 261. [CrossRef]
- 15. Selbach, C.; Soldánová, M.; Feld, C.K.; Kostadinova, A. Bernd Sures1. Hidden parasite diversity in a european freshwater system. *Sci. Rep.* **2020**, *10*, 2694. [CrossRef]
- Forrester, G.E.; Mccaffrey, M.T.; Terpis, K.X.; Lane, C.E. Using DNA barcoding to identify host-parasite interactions between cryptic species of goby (Coryphopterus: Gobiidae, Perciformes) and parasitic copepods (*Pharodes tortugensis*: Chondracanthidae, Cyclopoida). *Zootaxa* 2021, 5048, 99–117. [CrossRef] [PubMed]
- Cháves-González, L.E.; Morales-Calvo, F.; Mora, J.; Solano-Barquero, A.; Verocai, G.G.; Rojas, A. What lies behind the curtain: Cryptic diversity in helminth parasites of human and veterinary importance. *Curr. Res. Parasitol. Vector Borne Dis.* 2022, 2, 100094. [CrossRef] [PubMed]
- Buhler, K.J.; Snyman, L.P.; Fuglei, E.; Davidson, R.; Ptochos, S.; Galloway, T.; Jenkins, E. A circumpolar parasite: Evidence of a cryptic undescribed species of sucking louse, Linognathus sp., collected from Arctic foxes, *Vulpes lagopus*, in Nunavut (Canada) and Svalbard (Norway). *Med. Vet. Entomol.* 2023, *37*, 656–664. [CrossRef] [PubMed]
- 19. Miljević, M.; Rajičić, M.; Umhang, G.; Bajić, B.; Bjelić Čabrilo, O.; Budinski, I.; Blagojević, J. Cryptic species *Hydatigera kamiyai* and other taeniid metacestodes in the populations of small mammals in Serbia. *Parasit. Vectors* **2023**, *16*, 250.
- Brooks, D.R.; Hoberg, E.P. How will global climate change affect parasite-host assemblages? *Trends Parasitol.* 2007, 23, 571–574. [CrossRef]
- 21. Ndao, M. Diagnosis of parasitic diseases: Old and new approaches. Interdiscip. Perspect. Infect. Dis. 2009, 278246. [CrossRef]
- Dantas-Torres, F. Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. *Int. J. Parasitol. Parasites Wildl.* 2015, 4, e452–e461. [CrossRef] [PubMed]

- 23. Meurs, L.; Polderman, A.M.; Vinkeles Melchers, N.V.S.; Brienen, E.A.T.; Verweij, J.J.; Groosjohan, B.; Mendes, F.; Mechendura, M.; Hepp, D.H.; Langenberg, M.C.C.; et al. Diagnosing polyparasitism in a high-prevalence setting in Beira, Mozambique: Detection of intestinal parasites in fecal samples by microscopy and real-time PCR. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005310. [CrossRef]
- 24. Byers, J.E. Effects of climate change on parasites and disease in estuarine and nearshore environments. *PLoS Biol.* 2020, *18*, e3000743. [CrossRef] [PubMed]
- Paterson, R.A.; Poulin, R.; Selbach, C. Global analysis of seasonal changes in trematode infection levels reveals weak and variable link to temperature. *Oecologia* 2024, 204, 377–387. [CrossRef]
- McManus, D.P.; Bowles, J. Molecular genetic approaches to parasite identification: Their value in diagnostic parasitology and systematics. Int. J. Parasitol. Parasites Wildl. 1996, 26, 687–704. [CrossRef]
- Ahmed, M.; Singh, M.N.; Bera, A.K.; Bandyopadhyay, S.; Bhattacharya, D. Molecular basis for identification of species/isolates of gastrointestinal nematode parasites. *Asian Pac. J. Trop. Med.* 2011, *4*, 589–593. [CrossRef] [PubMed]
- Dzido, J.; Kijewska, A.; Rokicki, J. Selected mitochondrial genes as species markers of the Arctic *Contracaecum osculatum* complex. J. Helminthol. 2012, 86, 252–258. [CrossRef]
- Li, Y.; Chen, Z.; Liu, Z.; Liu, J.; Yang, J.; Li, Q.; Li, Y.; Cen, S.; Guan, G.; Ren, Q.; et al. Molecular identification of *Theileria* parasites of northwestern Chinese Cervidae. *Parasit. Vectors* 2014, 7, 225. [CrossRef]
- Olsson-Pons, S.; Clark, N.J.; Ishtiaq, F.; Clegg, S.M. Differences in host species relationships and biogeographic influences produce contrasting patterns of prevalence, community composition and genetic structure in two genera of avian malaria parasites in southern Melanesia. J. Anim. Ecol. 2015, 84, 985–998. [CrossRef]
- 31. Umbers, K.D.L.; Byatt, L.J.; Hill, N.J.; Bartolini, R.J.; Hose, G.C.; Herberstein, M.E.; Power, M.L. Prevalence and molecular identification of nematode and dipteran parasites in an Australian Alpine Grasshopper (*Kosciuscola tristis*). *PLoS ONE* **2015**, *10*, e0121685. [CrossRef]
- 32. Cai, P.; Gobert, G.N.; McManus, D.P. MicroRNAs in parasitic helminthiases: Current status and future perspectives. *Trends Parasitol.* **2016**, *32*, 71–86. [CrossRef] [PubMed]
- Mäser, P.; Brun, R. From Molecule to Drug. In *Molecular Parasitology. Protozoan Parasites and Their Molecules*; Walochnik, J., Duchêne, M., Eds.; Springer: Wien, Austria, 2016; pp. 491–507.
- Said, Y.; Gharbi, M.; Mhadhbi, M.; Dhibi, M.; Lahmar, S. Molecular identification of parasitic nematodes (Nematoda: Strongylida) in feces of wild ruminants from Tunisia. *Parasitology* 2017, 145, 901–911. [CrossRef] [PubMed]
- Kavunga-Membo, H.; Ilombe, G.; Masumu, J.; Matangila, J.; Imponge, J.; Manzambi, E.; Wastenga, F.; Ngoyi, D.M.; Van Geetruyden, J.P.; Muyembe, J.J. Molecular identification of *Plasmodium* species in symptomatic children of Democratic Republic of Congo. *Malar. J.* 2018, 17, 334. [CrossRef] [PubMed]
- Popinga, A.; Demastes, J.W.; Spradling, T.A.; Hafner, D.J.; Hafner, M.S. Host-parasite associations of the *Cratogeomys fumosus* species group and their chewing lice, *Geomydoecus. Therya* 2019, 10, 81–89. [CrossRef]
- Alburqueque, R.A.; Palomba, M.; Santoro, M.; Mattiucci, S. Molecular identification of zoonotic parasites of the genus *Anisakis* (Nematoda: Anisakidae) from fish of the Southeastern Pacific Ocean (Off Peru Coast). *Pathogens* 2020, 9, 910. [CrossRef] [PubMed]
- Abraham, D.; Graham-Brownb, J.; Carterc, D.; Grayc, S.A.; Hessa, J.A.; Makepeaceb, B.L.; Lustigman, S. Development of a recombinant vaccine against human onchocerciasis. Expert Rev. *Vaccines* 2021, 20, 1459–1470.
- Carrillo Bilbao, G.A.; Navarro, J.C.; Garigliany, M.-M.; Martin-Solano, S.; Minda, E.; Benítez-Ortiz, W.; Saegerman, C. Molecular identification of *Plasmodium falciparum* from captive non-human primates in the Western Amazon Ecuador. *Pathogens* 2021, 10, 791. [CrossRef] [PubMed]
- 40. Duflot, M.; Setbon, T.; Midelet, G.; Brauge, T.; Gay, M. A review of molecular identification tools for the opisthorchioidea. *J. Microbiol. Methods* **2021**, *187*, 106258. [CrossRef] [PubMed]
- Rollins, A.; Krupa, K.; Millward, G.; Piombino-Mascali, D.; Reinhard, K.; Kaestle, F. Molecular identification of parasites in an intestinal coprolite from a mummified religious dignitary of the Piraino Mother Church crypt, Sicily. J. Archaeol. Sci.-Rep. 2021, 38, 103022. [CrossRef]
- Pitaksakulrat, O.; Sithithaworn, P.; Kopolrat, K.Y.; Kiatsopit, N.; Saijuntha, W.; Andrews, R.H.; Petney, T.N.; Blair, D. Molecular identification of trematode parasites infecting the freshwater snail Bithynia siamensis goniomphalos in Thailand. *J. Helminthol.* 2022, 96, e49. [CrossRef]
- 43. Santacruz, A.; Barluenga, M.; Pérez-Ponce de León, G. The macroparasite fauna of cichlid fish from Nicaraguan lakes, a model system for understanding host–parasite diversification and speciation. *Sci. Rep.* **2022**, *12*, 3944. [CrossRef] [PubMed]
- 44. Strazdaitė-Žielienė, Ž.; Baranauskaitė, A.; Butkauskas, D.; Servienė, E.; Prakas, P. Molecular identification of parasitic Protozoa *Sarcocystis* in water samples. *Vet. Sci.* **2022**, *5*, 412. [CrossRef]
- Hui En Chan, A.; Thaenkham, U. From past to present: Opportunities and trends in the molecular detection and diagnosis of Strongyloides stercoralis. Parasit. Vectors 2023, 16, 123. [CrossRef] [PubMed]
- 46. Lazrek, Y.; Florimond, C.; Volney, B.; Discours, M.; Mosnier, E.; Houzé, S.; Pelleau, S.; Musset, L. Molecular detection of human *Plasmodium* species using a multiplex real time PCR. *Sci. Rep.* **2023**, *13*, 11388. [CrossRef]
- 47. Pietrzak-Makyła, B.; Korzeniewski, K.; Gładysz, P.; Lass, A. Detection and molecular characterization of *Blastocystis* species in Polish soldiers stationed in the Republic of Kosovo. *Int. J. Mol. Sci.* **2023**, 24, 14100. [CrossRef]
- 48. Bártová, E.; Marková, J.; Sedláčková, J.; Band'ouchová, H.; Račka, K. Molecular detection of *Toxoplasma gondii*, *Neospora caninum* and *Encephalitozoon* spp. in vespertilionid bats from Central Europe. *Int. J. Mol. Sci.* **2023**, 24, 9887. [CrossRef] [PubMed]

- Aghazadeh, M.; Jones, M.; Perera, S.; Nair, J.; Tan, L.; Clark, B.; Curtis, A.; Jones, J.; Ellem, J.; Olma, T.; et al. The application of 3base[™] technology to diagnose eight of the most clinically important gastrointestinal protozoan infections. *Int. J. Mol. Sci.* 2023, 24, 13387. [CrossRef]
- 50. Dąbrowska, J.; Sroka, J.; Cencek, T. Investigating *Cryptosporidium* spp. using genomic, proteomic and transcriptomic techniques: Current progress and future directions. *Int. J. Mol. Sci.* **2023**, 24, 12867. [CrossRef]
- 51. Morán, P.; Serrano-Vázquez, A.; Rojas-Velázquez, L.; González, E.; Pérez-Juárez, H.; Hernández, E.G.; de los Angeles Padilla, M.; Zaragoza, M.E.; Portillo-Bobadilla, T.; Ramiro, M.; et al. Amoebiasis: Advances in diagnosis, treatment, immunology features and the interaction with the intestinal ecosystem. *Int. J. Mol. Sci.* 2023, 24, 11755. [CrossRef]
- Perrone, A.E.; Pinillo, M.; Rial, M.S.; Fernández, M.; Milduberger, N.; González, C.; Bustos, P.L.; Fichera, L.E.; Laucella, S.A.; Albareda, M.C.; et al. *Trypanosoma cruzi* secreted cyclophilin *Tc*CyP19 as an early marker for trypanocidal treatment efficiency. *Int. J. Mol. Sci.* 2023, 24, 11875. [CrossRef]
- 53. Jamabo, M.; Mahlalela, M.; Edkins, A.L.; Boshoff, A. Tackling sleeping sickness: Current and promising therapeutics and treatment strategies. *Int. J. Mol. Sci.* 2023, 24, 12529. [CrossRef] [PubMed]
- Palkumbura, P.G.A.S.; Mahakapuge, T.A.N.; Wijesundera, R.R.M.K.K.; Wijewardana, V.; Kangethe, R.T.; Rajapakse, R.P.V.J. Mucosal immunity of major gastrointestinal nematode infections in small ruminants can be harnessed to develop new prevention strategies. *Int. J. Mol. Sci.* 2024, 25, 1409. [CrossRef] [PubMed]

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