Novel β-tubulin mutations and haplotype sweeps characterize benzimidazole resistance in *Parascaris univalens*

Benzimidazole (BZ) resistance in *Parascaris univalens*, a major equine parasite, poses an emerging threat to parasite control. Unlike strongyle nematodes, *Parascaris* spp. lack the canonical β-tubulin mutations (F167Y, E198A, F200Y) associated with BZ resistance in other parasite species. To further investigate the molecular mechanisms behind BZ-resistance in *Parascaris* spp. two complementary strategies were applied in this study. First, all seven β-tubulin isotypes (A–G) using pooled eggs from a BZ-resistant (45% efficacy) and fully susceptible farm sequenced. Deep amplicon sequencing generated 2.3 million PacBio HiFi reads spanning complete gene loci. Read- and cluster-based pipelines identified variants, phased haplotypes, and selection signatures. Second, to distinguish genetic from transcriptional mechanisms, β-tubulin expression was analyzed in adult worms following 24-hour *in vitro* thiabendazole exposure using RNA-seq. None of the canonical BZ-resistance SNPs were detected at codons 167, 198, or 200. Instead, three novel amino acid substitutions showed strong resistance associations in the resistant isolate: V315M (OR > 8400, $p < 0.001$) and F167S (OR > 2100, $p < 0.001$) in *Pun-bt-B*, and Q447P (OR = 3.94, $p < 0.001$) in *Pun-bt-C*. *Pun-bt-B* exhibited an extreme selective sweep with 9.5-fold haplotype diversity reduction (n_{95}: 2 vs 19) and near fixation of a single resistant haplotype, representing a selective sweep. *Pun-bt-C* showed moderate consolidation (2.3-fold) with enrichment of two resistant meta-haplotypes, while *Pun-bt-E* displayed significant depletion (30-fold) of susceptible-associated haplotypes. Expression analysis confirmed high basal transcription of *Pun-bt-A* and *Pun-bt-B*, but no consistent BZ-induced changes, indicating genetically encoded resistance rather than transcriptionally regulated. It can therefore be concluded that BZ resistance in *P. univalens* is defined by selective sweeps and novel β-tubulin mutations (V315M, Q447P) rather than canonical SNPs. These findings established distinct ascarid-specific resistance mechanisms

and identify candidate molecular markers for future diagnostics and surveillance. These findings indicate that a broader genome-based monitoring approaches are recommended over traditional single-site genotyping.