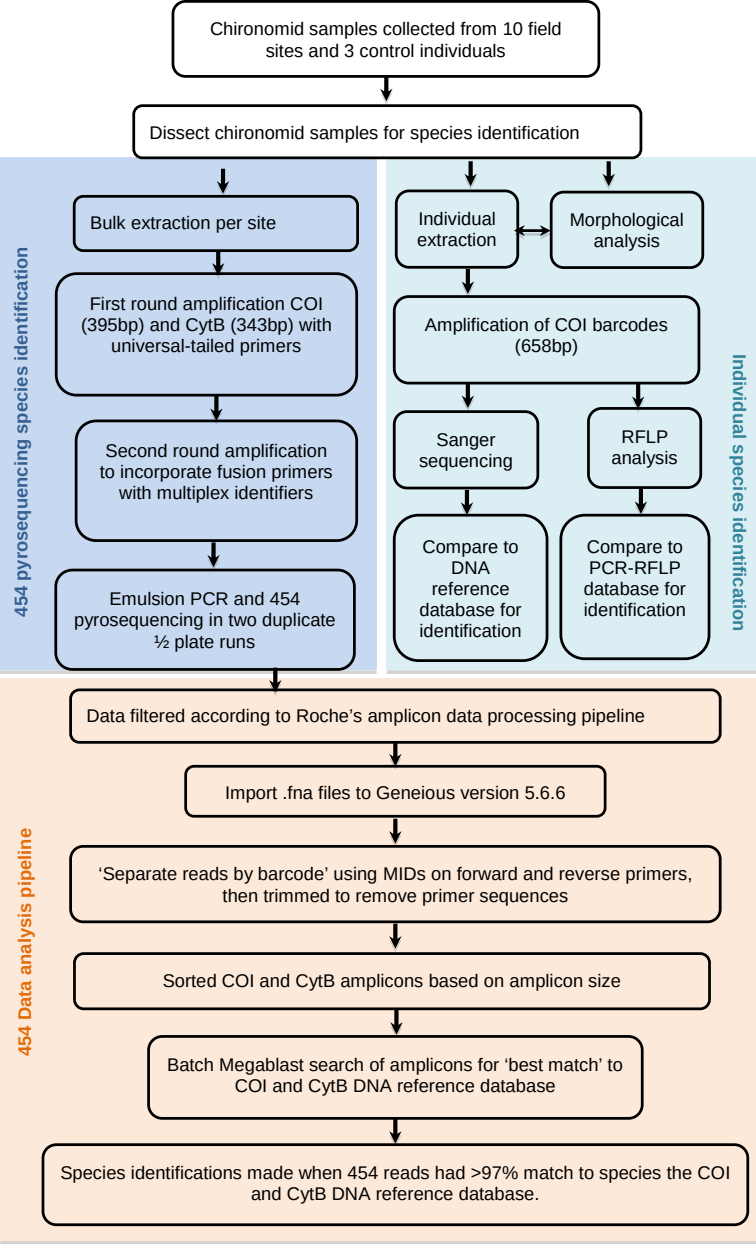
Chironomid samples collected from 10 field sites and 3 control individuals
Dissect chironomid samples for species identification
454 pyrosequencing species identification
Bulk extraction per site
First round amplification COI (395bp) and CytB (343bp) with universal-tailed primers
Second round amplification to incorporate fusion primers with multiplex identifiers
Emulsion PCR and 454 pyrosequencing in two duplicate ½ plate runs
Individual species identification
Individual extraction
Morphological analysis
Amplification of COI barcodes (658bp)
Sanger sequencing
RFLP analysis
Compare to DNA reference database for identification
Compare to PCR-RFLP database for identification
454 Data analysis pipeline
Data filtered according to Roche's amplicon data processing pipeline
Import .fna files to Geneious version 5.6.6
'Separate reads by barcode' using MIDs on forward and reverse primers, then trimmed to remove primer sequences
Sorted COI and CytB amplicons based on amplicon size
Batch Megablast search of amplicons for 'best match' to COI and CytB DNA reference database
Species identifications made when 454 reads had >97% match to species the COI and CytB DNA reference database.