

Table A2: Feature Comparison between PARAFISH and PMiFish

Feature / Functionality	PARAFISH	PMiFish (based on available descriptions)
<i>Core Architecture</i>		
Primary Language/Framework	Python (Dash web framework)	Perl
Interface	Web-based Graphical User Interface (GUI)	Command-Line Interface (CLI)
Installation/Accessibility	Web application (local/server deployment with Python dependencies)	Local installation of Perl scripts & dependencies
Modularity (Start at any step)	Yes, highly modular	Primarily full pipeline execution from scripts
<i>Workflow Stages</i>		
Quality Control	FastQC (detailed HTML reports)	Basic quality filtering (e.g., via USEARCH)
Read Trimming	Trimmomatic (adapter & quality trimming)	Basic trimming (primers, low quality)
Paired-End Read Merging	PEAR	Yes (e.g., via USEARCH <code>fastq_mergepairs</code> )
Primer Removal	Integrated within Trimmomatic/PEAR steps	Yes (custom scripts / USEARCH)
OTU Clustering / ASV Inference	VSEARCH (OTU clustering), DADA2 (ASV inference), Kraken2 (direct taxonomic classification)	USEARCH (OTU clustering, denoising for ZOTUs/ASVs)
Chimera Detection	Not an explicit user-configurable step (VSEARCH internal checks)	Yes (e.g., UCHIME within USEARCH workflow)
<i>Taxonomic &amp; Phylogenetic Analysis</i>		
Taxonomic Assignment Tools	BOLD (via BOLD-igger), NCBI, SILVA, PR2	USEARCH <code>usearch_global</code> or similar
Primary Reference Database(s)	BOLD (via BOLD-igger)	PMiFish DB, NCBI nt (customizable)
Multiple Sequence Alignment	MAFFT	No (suggests external tools like MEGA X)
Phylogenetic Tree Inference	FastTree2 (approximate ML), RAxML-NG (rigorous ML + Bootstrapping)	No (suggests external tools like MEGA X)
<i>Usability &amp; Output</i>		
Interactive Visualizations	FastQC reports, Phylogenetic trees (Cytoscape.js)	Primarily text files/tables; relies on external tools for visuals
Result Output	Downloadable files per step, comprehensive logs	Output files (FASTA, tables), script logs
Pipeline Orchestration	Python (Dash framework) orchestrates backend tools	Main Perl script calls other scripts/tools
Post-processing Summary	Downloadable results, interactive visualizations	Summary tables, FASTA files per family
<i>Focus &amp; Strengths</i>		
Focus & Strengths	Intuitive web UI, BOLD-igger integration, full phylogenetic pipeline, interactive tree viz.	Specialized for MiFish protocol, established USEARCH-centric workflow