Item S1. Additional information about calculations and construction of Figure 3.

To estimate the relative sequence similarity and number of mismatching nucleotides in long-read and short-read rRNA gene sequence data, we downloaded all bacterial rRNA sequences >4000 bases from GenBank and all fungal rRNA and genomic sequences >5000 bases from both GenBank and UNITE. From each species, we took only the longest fragment containing rRNA genes to avoid sampling the same isolate. We trimmed the 16S/18S-ITS-23S/28S fragments using the primers 27F and 23SR for bacteria and SSU3a and TW13 for fungi using the cutadapt software (https://bio.tools/cutadapt). For fungi, this partial rRNA gene fragment was the longest, for which reads from >500 species could be obtained. For fungi, we used all 685 reads, but for bacteria we randomly selected 1418 reads. For both groups, the 16S/18S-ITS-23S/28S fragments were further separated into 11 shorter fragments comprising medium-size and typical short markers of ITS (only in fungi) and 16S/18S, most of which correspond to the commonly used primers and DNA markers using in DNA barcoding and metabarcoding studies (Table). Across all reads and DNA markers, the number of matching bases were computed and expressed as the number of mismatches and per cent differences. The occasional >80% sequence differences in fungal ITS1 and ITS2 regions were converted to 80.0% to avoid excessively large values. Separate values were calculated for congeneric pairs. For bacteria, 1,028,572 domain-level pairs (n=1418 species) and 17,063 genus-level pairs (n=1106 species, i.e. 312 species had no congeneric pairs) were used. For fungi, 324,415 kingdom-level pairs (n=685 species) and 756 conspecific pairs (n=268 species; i.e. 488 species with no congeneric pairs) were used.

Differences in the number of mismatches and sequence dissimilarity (mismatches per alignment length) among 12 marker fragments were compared using 99.990% confidence intervals (corresponds to $P_{adjusted}$=0.0011) when accounting for multiple comparisons among closest ranking neighbours (or $P_{adjusted}$=0.0066 when considering all unique pairwise comparisons). We conservatively used the number of species rather than pairwise comparisons as replicates to avoid overestimating the number of *independent* comparisons.

Table. Primers and their sequences used for delimiting DNA fragments.

Marker	Fwd primer	Fwd primer sequence	Rev primer	Rev primer sequence
Bacteria				
16S-ITS-23S	27F	AGAGTTTGATCMTGGCTCAG	23SR	TYTRAACCCARCTCRCGTDCC
16S+ITS	27F	AGAGTTTGATCMTGGCTCAG	23SR	YGCCARGGCATCCRCC
ITS+23S	1507F	GGTGAAGTCGTAACAAGGTA	23SR	TYTRAACCCARCTCRCGTDCC
16S	27F	AGAGTTTGATCMTGGCTCAG	1492R	TACCTTGTTACGACTT
ITS	1507F	GGTGAAGTCGTAACAAGGTA	28F	YGCCARGGCATCCRCC
23S	129F	CYGAATGGGRVAACC	23SR	TYTRAACCCARCTCRCGTDCC
16S V1-V2	27F	AGAGTTTGATCMTGGCTCAG	336R	ACTGCTGCSYCCCGTAGGAGTCT
16S V3-V4	341F	CCTACGGGNSGCWGCAG	806RB	GGACTACNVGGGTWTCTAAT
16S V4	515FB	GTGYCAGCMGCCGCGGTAA	806RB	GGACTACNVGGGTWTCTAAT
16S V4-V5	515FB	GTGYCAGCMGCCGCGGTAA	926R	CCGYCAATTYMTTTRAGTTT
16S V7-V9	1100F	GYAACGAGCGCAACCC	1492R	TACCTTGTTACGACTT
23S D1	129F	CYGAATGGGRVAACC	457R	CCTTTCCCTCACGGTACT
Fungi				
18S-ITS-28S	SSU3a	CAGCAGCCGCGGTAATTC	TW13	GGTCCGTGTTTCAAGACG
18S+ITS	SSU3a	CAGCAGCCGCGGTAATTC	ITS4ngsUni	CCTSCSCTTANTDATATGC
ITS+28S	ITS1catta	ACCWGCGGARGGATCATTA	TW13	GGTCCGTGTTTCAAGACG
18S	SSU3a	CAGCAGCCGCGGTAATTC	NS8a	CCTCTAAATGACCRAGTTTG
ITS	ITS1catta	ACCWGCGGARGGATCATTA	ITS4ngsUni	CCTSCSCTTANTDATATGC
28S	LR0R	ACCCGCTGAACTTAAGC	TW13	GGTCCGTGTTTCAAGACG
18S V4	SSU3a	CAGCAGCCGCGGTAATTC	NS20	CGTCCCTATTAATCATTACG
18S V9	ITS9MUNngs	GTACACACCGCCCGTCG	NS8a	CCTCTAAATGACCRAGTTTG
ITS1	ITS1catta	ACCWGCGGARGGATCATTA	ITS2ngs	TTYRCKRCGTTCTTCATCG
ITS2	gITS7ngs	GTGARTCATCRARTYTTTG	ITS4ngsUni	CCTSCSCTTANTDATATGC
28S D1	LR0R	ACCCGCTGAACTTAAGC	LF402	TTCCCTTTYARCAATTTCAC
28S D2	LF402Fmix	GTGAAATTGYTRAAAGGGAA	TW13	GGTCCGTGTTTCAAGACG