Diversity, transfer potential, and transcriptional activity of virus-carried antibiotic resistance genes in global estuaries

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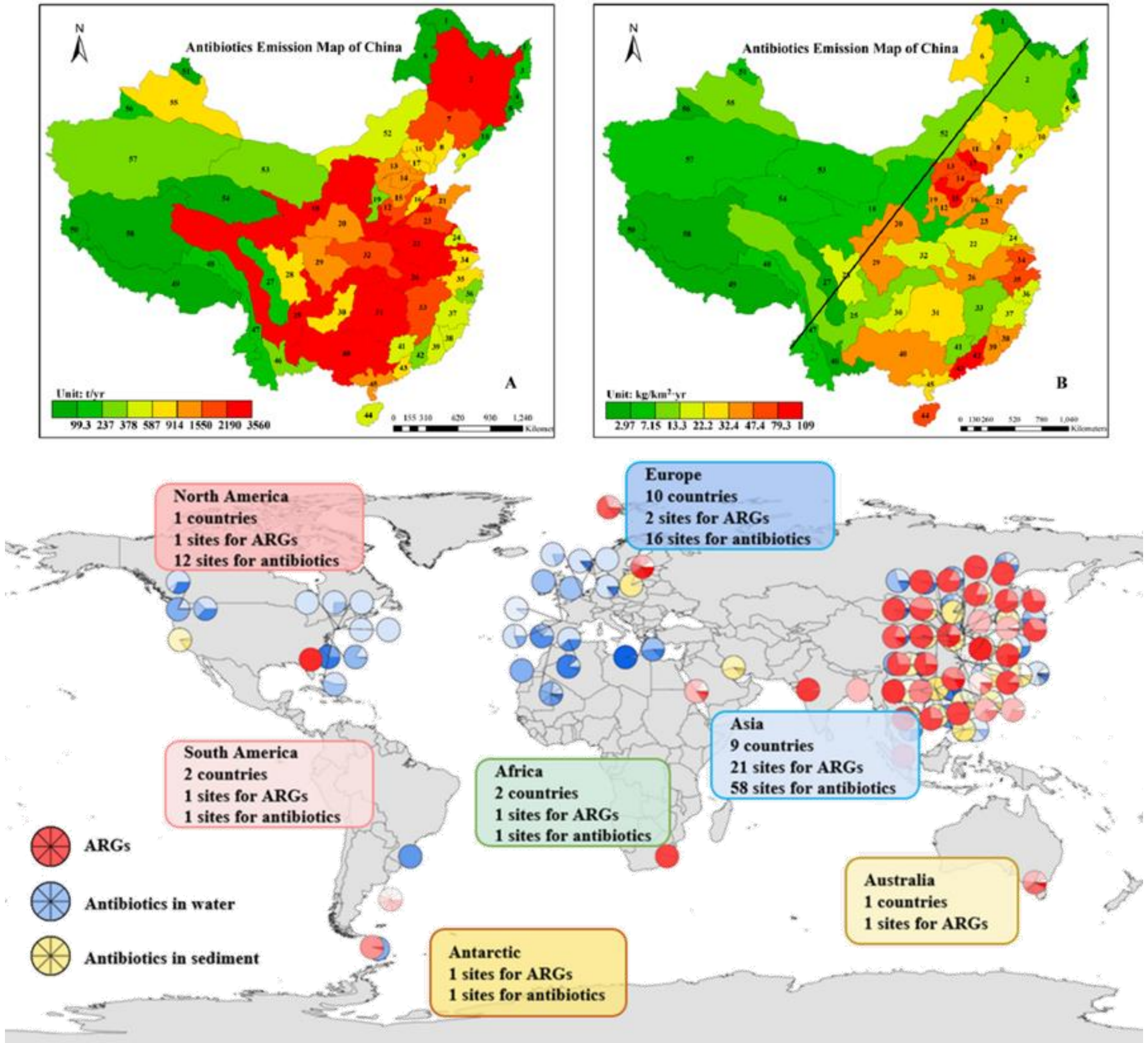
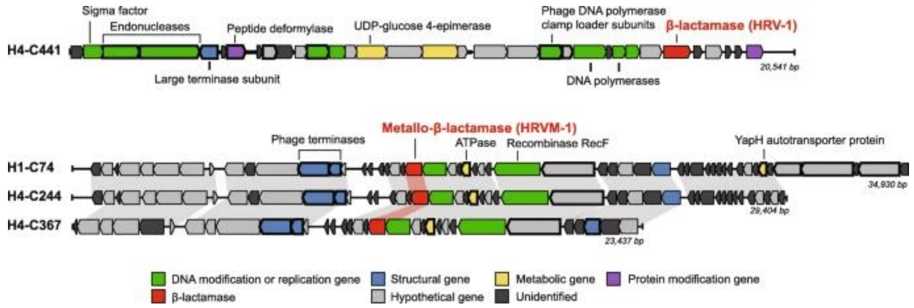
Introduction

Severe antibiotic pollution in estuaries



Antibiotic emissions in Chinese river basins and global estuaries

Contributions of viruses to ARG spread



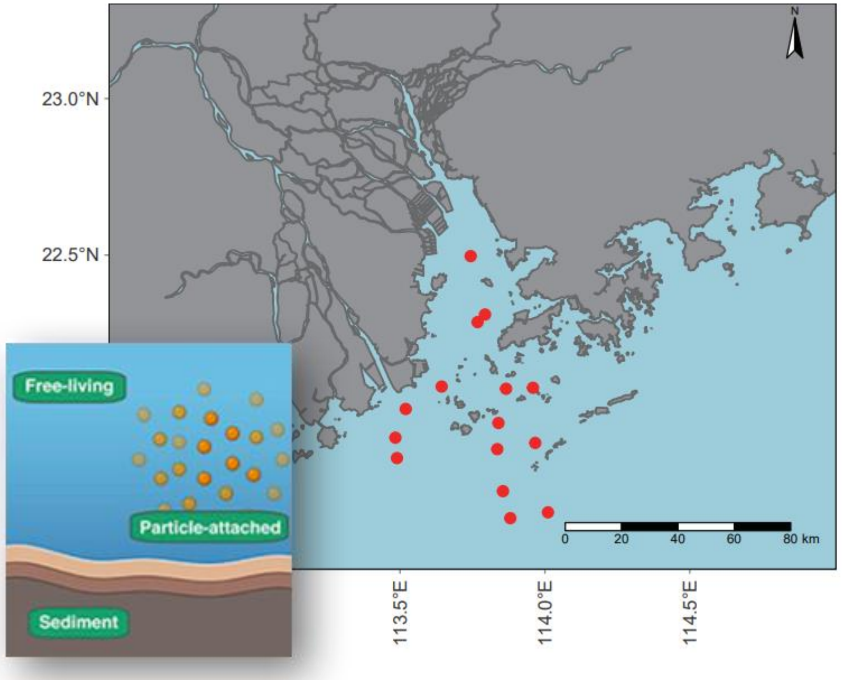
Viruses as vectors of ARGs

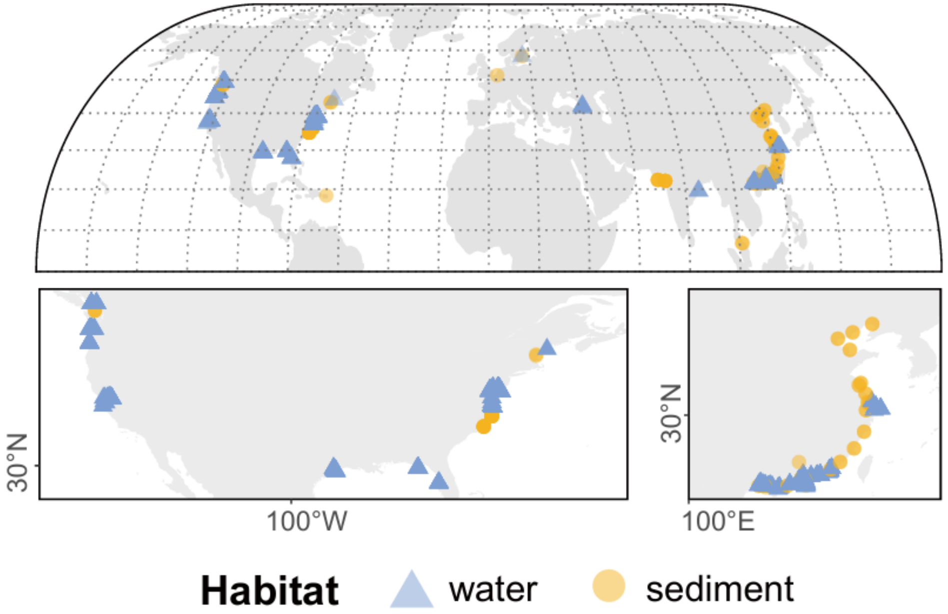
Viral transduction can mediate horizontal ARG transfer

Studying viral ARGs is key to assessing ARG transmission risk in estuaries.

(Zhang *et al*., Environ. Sci. Technol. 2015; Zheng *et al*., Sci. Total Environ. 2021; Zhang *et al*., Front. Microbiol. 2022)

Construction of the Global Estuarine Virome Dataset (GEV)





Pearl River Estuary

60 samples

Water: 23; Particle: 23; Sediment: 14

Metagenomes, metatranscriptomes

Physicochemical factors

Global Estuaries

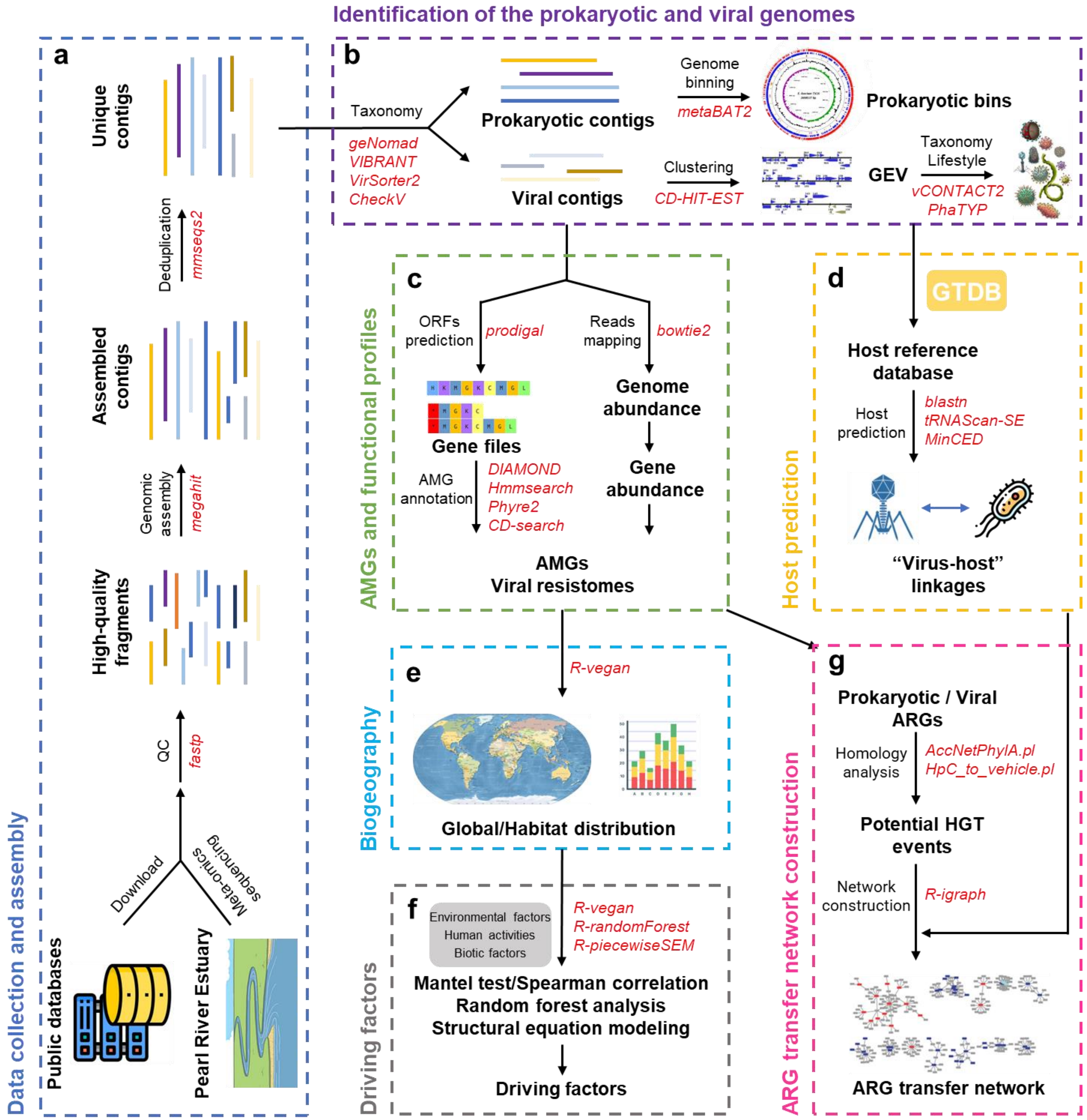
624 samples

73 estuaries, 9 countries, 4 continents

Viromes, metagenomes, metatranscriptomes

Physicochemical factors

Bioinformatic workflow



Bioinformatic workflow

Scientific issues

- How vARGs contribute to the estuarine antibiotic resistome in terms of diversity, abundance, transmission, and expression?
- What factors influence these vARG characteristics?

Overview of the GEV dataset

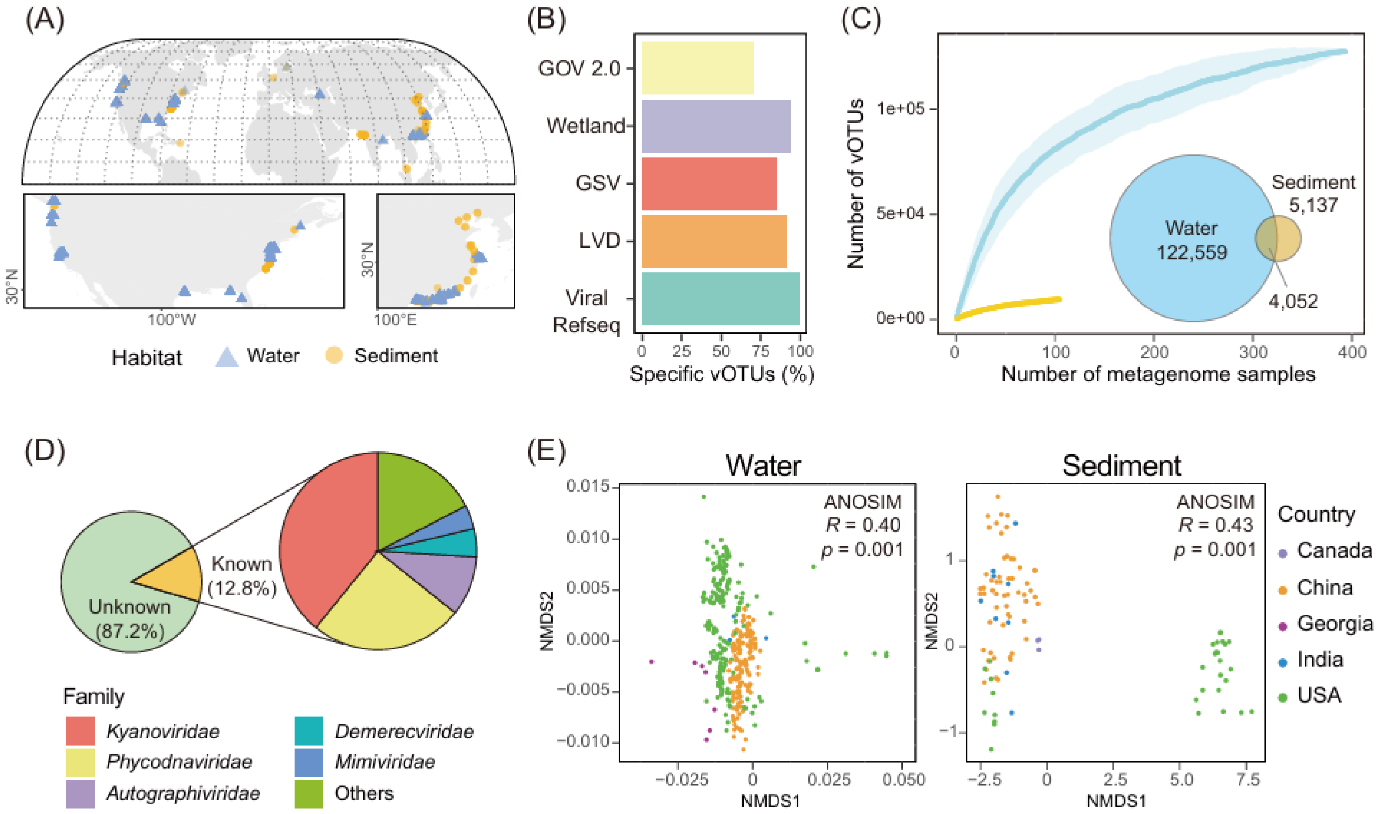


Figure 1 Overview of the global estuarine virome (GEV) database.

Estuarine viromes are novel and diverse

- 146,207 viral contigs (≥ 10 kbp) ;
- 136,443 vOTUs;
- Only 12.8% of the estuarine vOTUs could be classified into viral families;
- More than 70% of the vOTUs did not share clusters with other databases;
- The diversity of estuarine viromes remains largely unexplored.

Estuarine viruses carry diverse vARGs

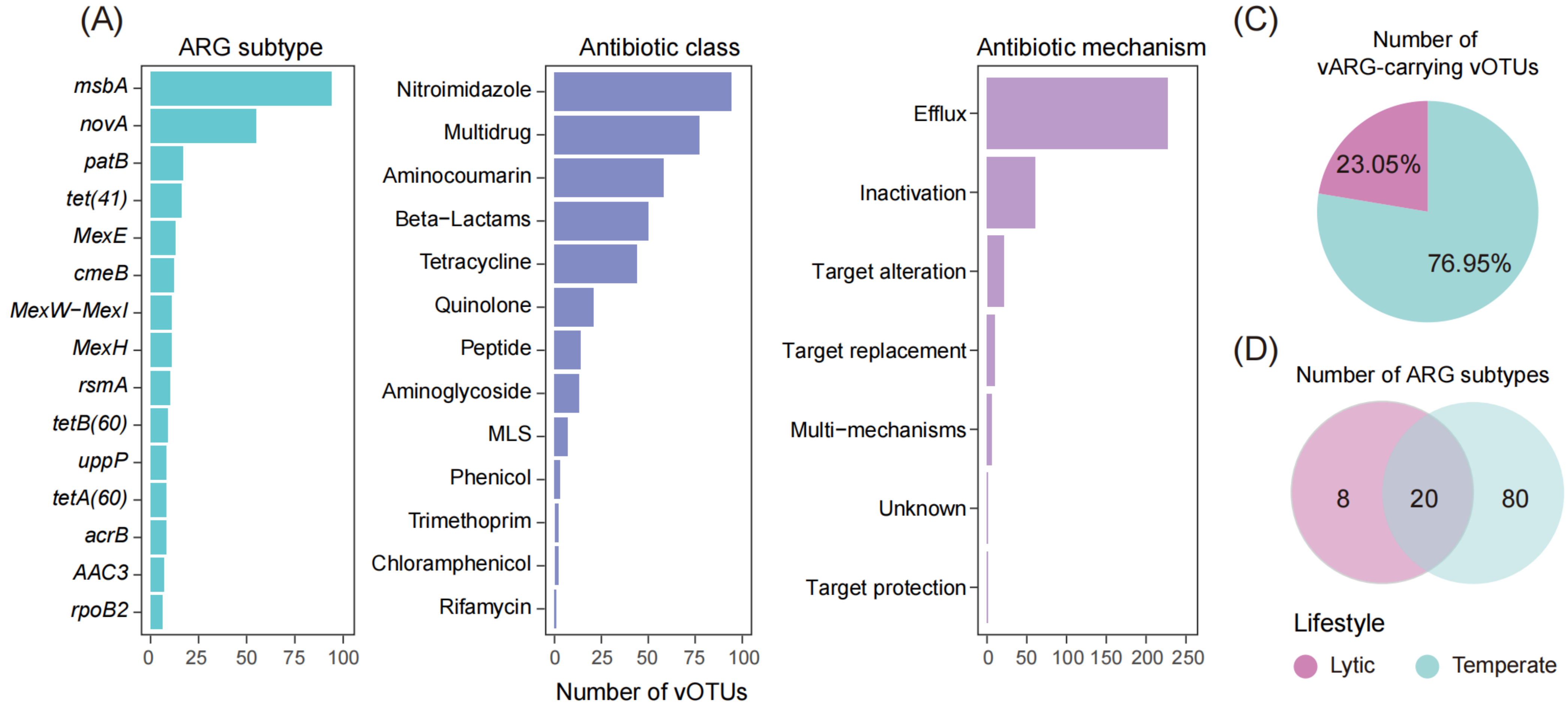


Figure 2 Abundant and diverse vARGs in estuarine environments.

- 0.2% of estuarine vOTUs carrying ARGs;
- vARGs encompassing 108 subtypes of ARGs that could confer resistance to 13 classes of antibiotics through six distinct resistance mechanisms

Estuarine viruses carry diverse vARGs

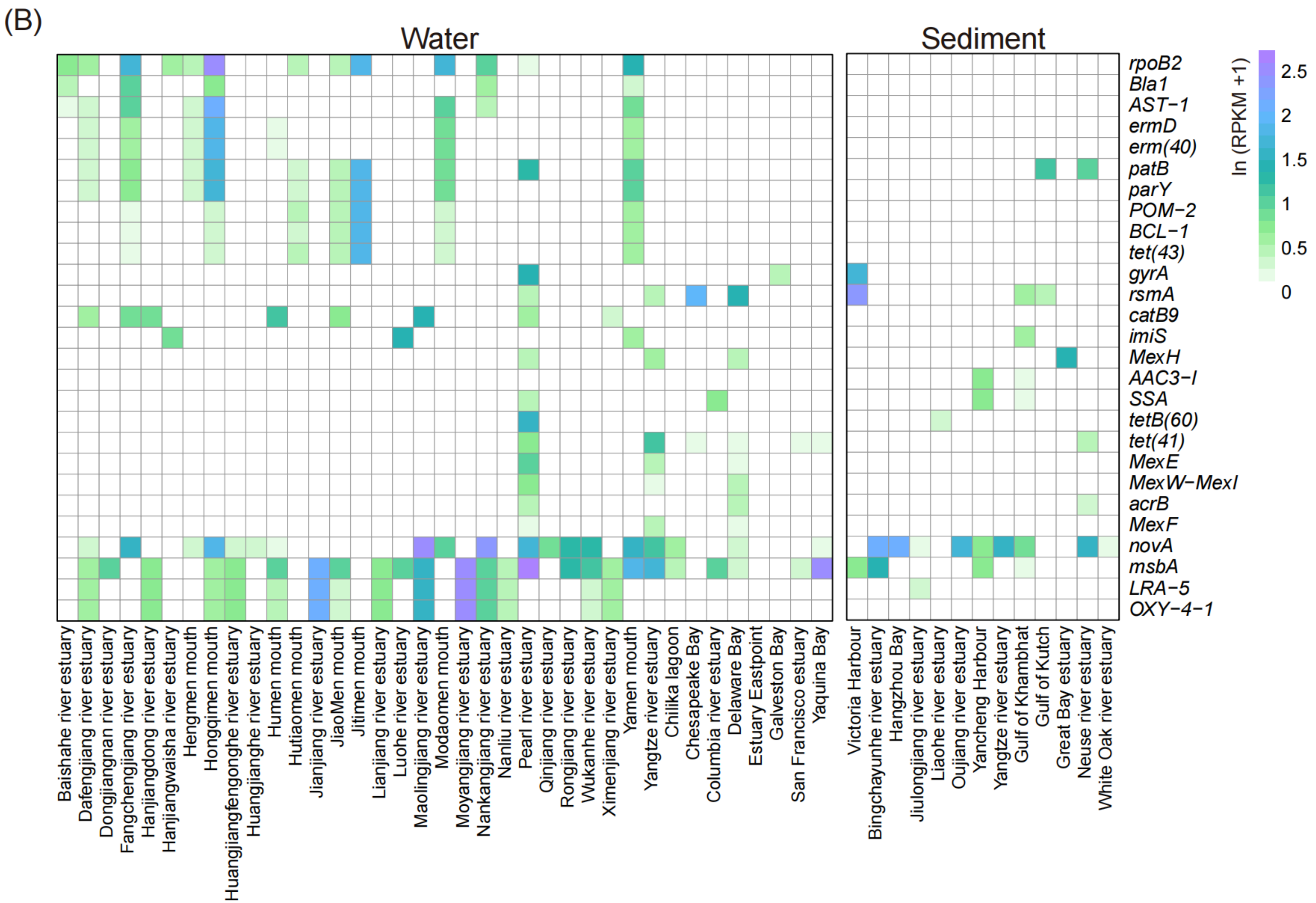


Figure 2 Abundant and diverse vARGs in estuarine environments.

- 25 vARG have also been ranked as high health risk (Q1);
- vARGs contributed more than 10% of the total ARG abundance in 17 samples.

Estuarine vARGs target a broad range of hosts

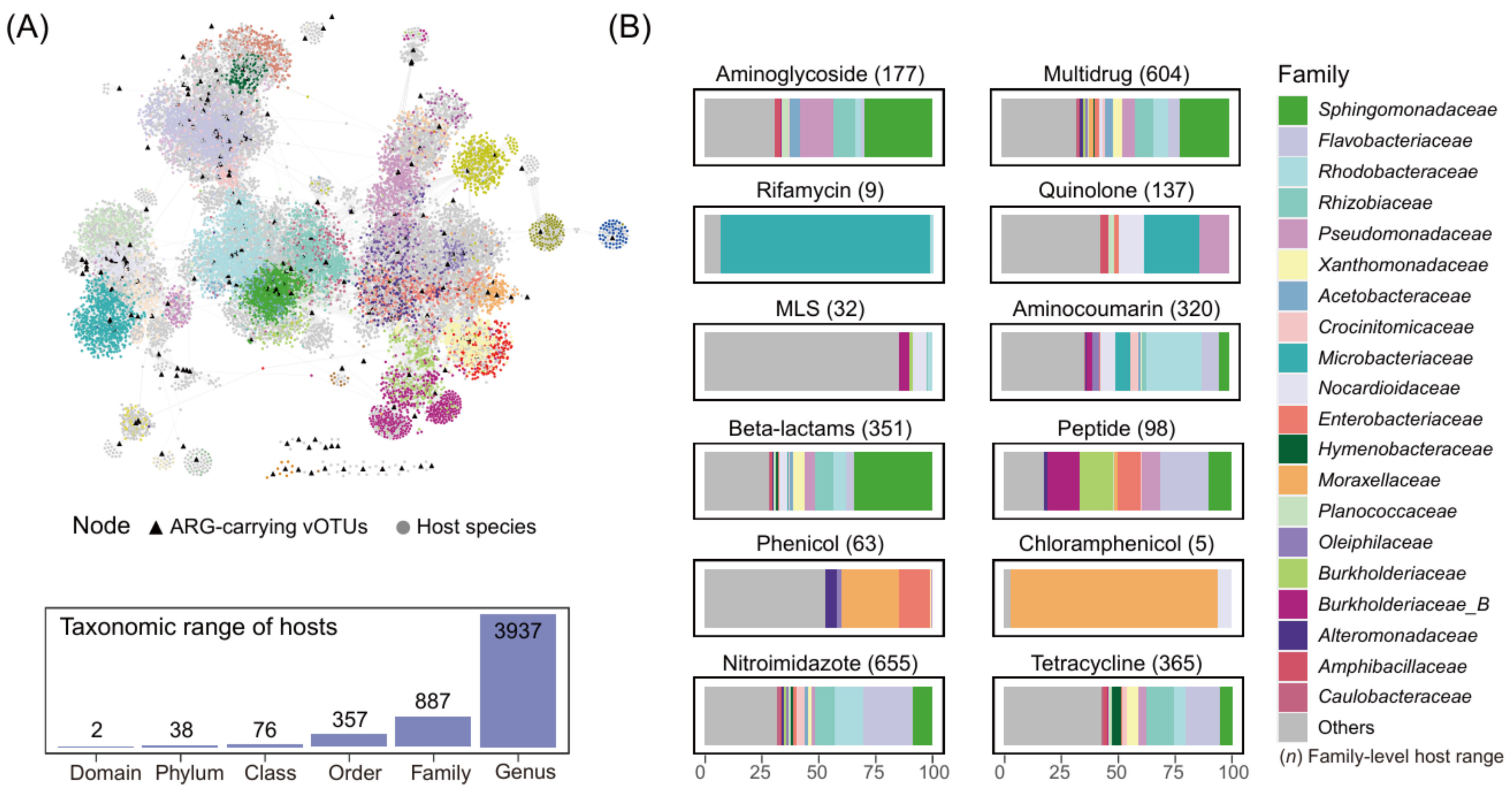


Figure 3 Predicted virus-host linkages of the ARG-carrying vOTUs in the GEV.

- 83% of ARG-carrying vOTUs were assigned to potential hosts;
- Hosts spanned two archaeal and 36 bacterial phyla;
- vOTUs with higher vARG density tended to interact with more prokaryotic taxa;
- Viral communities carrying specific vARGs could target over a hundred prokaryotic families.

Estuarine vARGs exhibit significant transmission potential

- 443 potential ARG transfers;
- 178 vARGs, 112 vOTUs, 235 host MAGs;
- Two or more different ARGs were involved within one specific virus-host linkage (red);
- Some viruses carrying different ARGs were linked to the same host;
- Potential pathogens were involved in nearly 2.3% of ARG transfer events。

Figure 4 Virus-mediated ARG transfers in the estuarine environments.

vARGs are actively expressed in estuarine environments

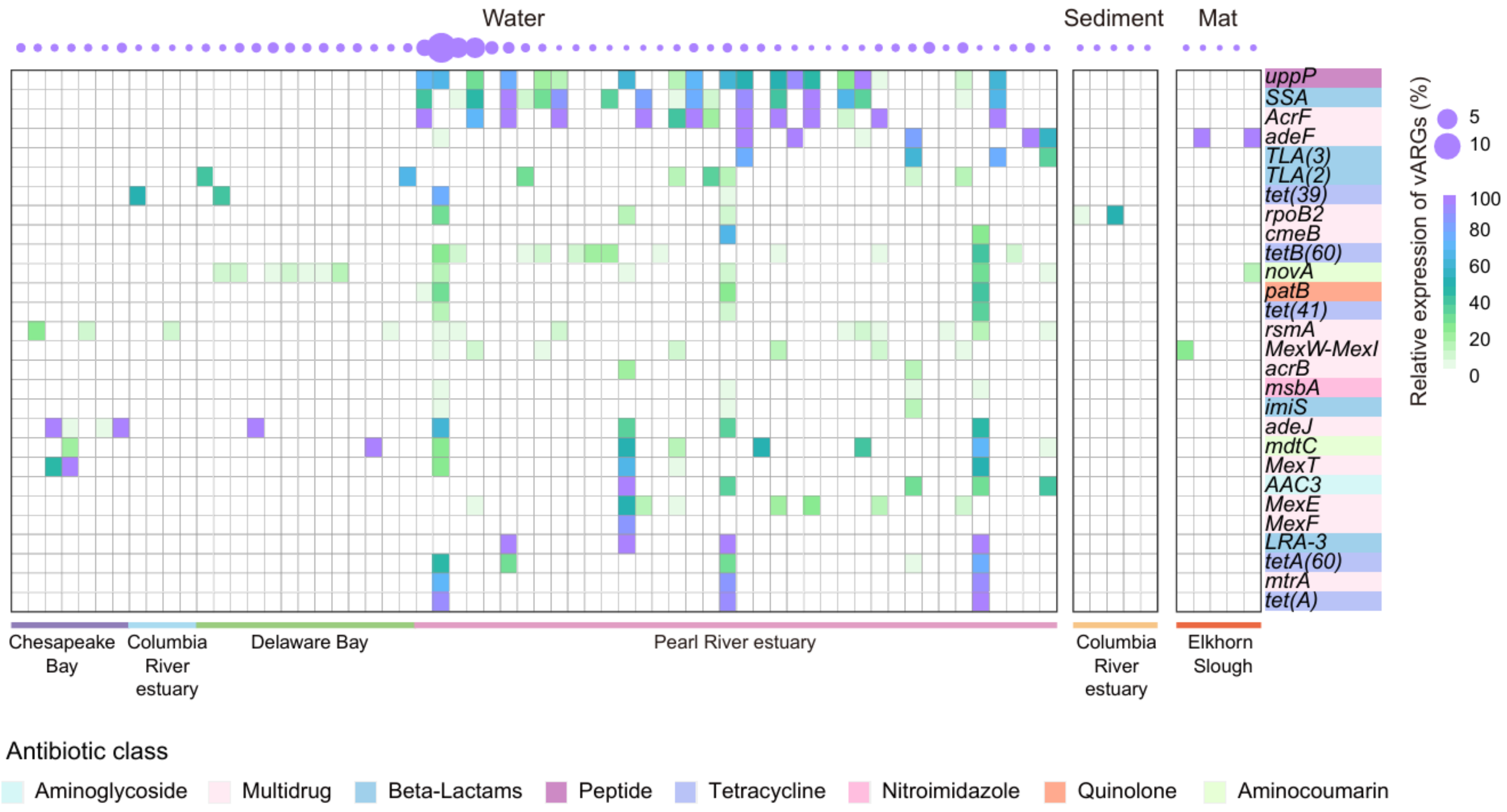


Figure 5 Contributions of vARGs to the overall expression of estuarine antibiotic resistome.

- Over half (51%) of the vARGs were active in the estuaries;
- *msbA* was the most active vARG;
- Multiple vARGs were expressed simultaneously in almost half of the samples;
- Over 5% of total ARG expression was contributed by vARGs in three samples;
- The relative expressions of some vARGs exceeded their prokaryotic counterparts.

Elevated temperature and human activities may raise vARG risks

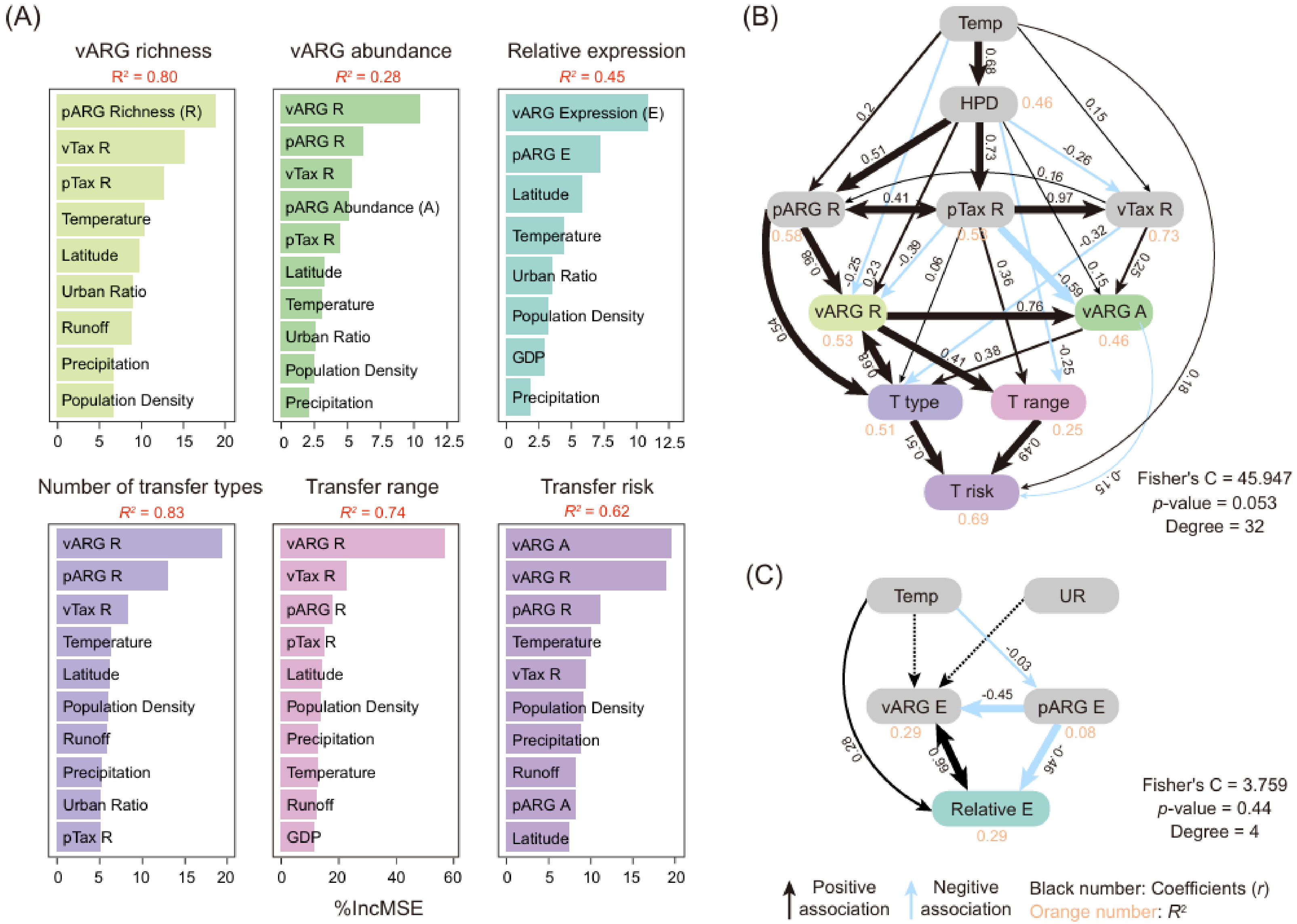
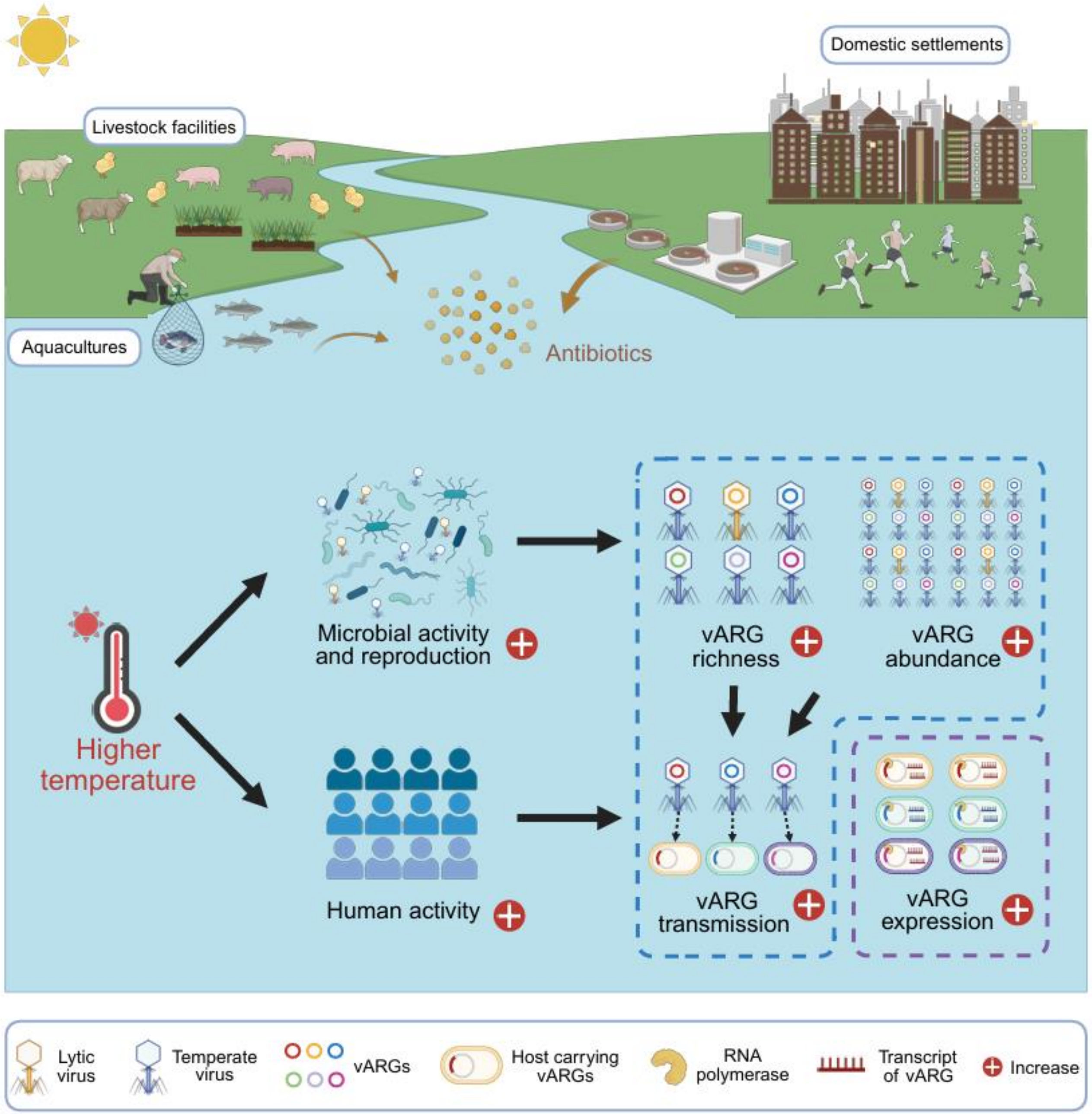


Figure 6 Drivers of the vARG richness, abundance, expression, and transmission in estuarine water.

- Biotic factors: the richness of pARGs and the whole viral (vTax) and prokaryotic (pTax) communities;
- Abiotic factors: temperature, latitude, population density, and urban ratio;
- Temperature and human activities exhibited both direct and indirect effects on vARGs.

Elevated temperature and human activities may raise vARG risks



Two main explanations

- Higher temperatures could increase prokaryotic metabolism and reproduction in estuaries, and in turn, boost viral replication and the proliferation, transmission, and expression of vARGs;
- More antibiotic residues entering into the tropical/subtropical estuaries, which may increase the selective advantage of ARG-carrying microorganisms.

Figure 7 Conceptual model depicting the potential changes of vARG characteristics under global warming.

Summary

- Estuarine viruses, particularly temperate viruses, carried abundant and diverse ARGs, and over half of the vARGs were actively expressed in estuarine environments.

- vARG-carrying viruses targeted a wide range of prokaryotes and likely facilitated horizontal gene transfer of vARGs between viruses and prokaryotes, supported by sequence similarity and minimal evolutionary distance analyses.

- Elevated temperatures and coastal human activities could significantly increase the richness, abundance, transmission risk, and relative expression of estuarine vARGs.

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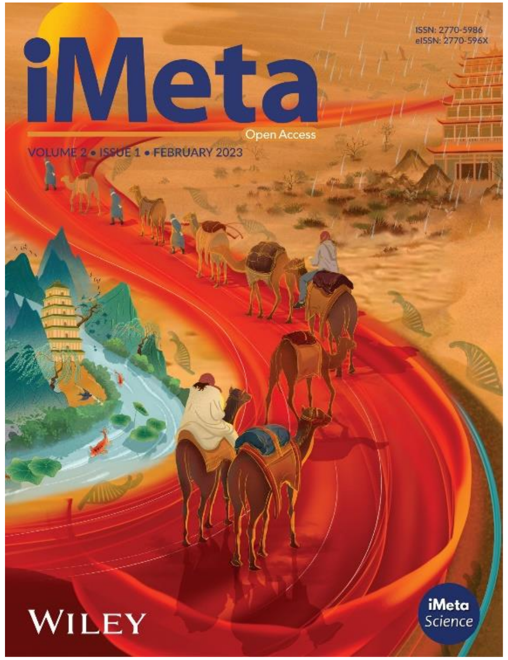

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