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Article type : Opinion

More than meets the eye: the barrier effect of the Yangtze River outflow

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Keywords: East China Sea, marine phylogeography, freshwater outflow barrier, northwestern Pacific, Changjiang River, marine population connectivity

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Abstract

The Yangtze (Changjiang) River enters the East China Sea with huge annual freshwater and sediment deposits. This outflow, known as the Changjiang Diluted Water (CDW), causes

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/mec.14235

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striking ecological gradients that potentially shape coastal species' distribution and differentiation. The CDW has long been rendered as a marine biogeographic boundary separating cold-temperature and warm-water faunas, but it remains unclear whether and to what extent it acts as an intraspecific barrier. Here we synthesize published phylogeographic studies related to the CDW to address these issues. We find that the influence of the CDW on population differentiation is taxonomically variable, and even congeneric species may respond differently. In studies that claim the CDW is a phylogeographic barrier, the underlying assumptions explaining observed genetic breaks are sometimes incorrect, and some may have misinterpreted results due to conceptual confusion or insufficient geographical sampling. After excluding these studies, the remaining ones generally show shallow genetic divergence but significant population structure for coastal species across the CDW, suggesting that the CDW has not been a historically persistent barrier, but rather has acted as a filter within some species recently, probably after the last glacial maximum.

Introduction

Physical barriers that restrict the dispersal of individuals between populations may produce evolutionary partitions and maintain distinct biotic assemblages limited to either side of the barrier (Briggs & Bowen 2013; De Bruyn *et al.* 2005; Taylor & Hellberg 2006). Accurately identifying these barriers and clarifying their biological effects are critical steps towards understanding regional biogeography and evolutionary history (Holt *et al.* 2013).

The Yangtze River (also known as the Changjiang, Fig. 1A), the largest in Asia and the third longest in the world, represents a significant environmental factor that influences biogeographic patterns of the biota in the northwestern Pacific (Beardsley *et al.* 1985). It enters the East China Sea (ECS) with about $9 \times 10^{11} \text{ m}^3$ of freshwater discharge and 4.8×10^8 tons of mud deposit annually (Liu *et al.* 2007a; Su & Yuan 2005). This huge freshwater

outflow, named the Changjiang diluted water (CDW), is defined by temperature-salinity relationships based on Gong *et al.* (1996) with temperatures ≥ 23 °C and salinity ≤ 31 PSU.

The CDW causes significant shifts in various ecological and environmental parameters in the ECS, including hydrography, topography, and nutrient concentrations (e.g. Liu *et al.* 2010; Wang *et al.* 2003; Wu *et al.* 2010). The strong influence of water property gradients such as salinity and temperature (Fig. 1B, C, respectively) around the river mouth are considered to limit the movement of individuals and constrain regional assemblages of species (Liu 2013; Yu *et al.* 2014). The CDW is thus rendered as a biogeographic boundary along the coast of mainland China (Liu 2013), separating the North Pacific Temperate Biotic Region (characterized by cold temperate fauna) from the Indo-West Pacific Warm-water Biotic Region (characterized by tropical/subtropical fauna) (shown as regions I and II respectively in Fig. 1A).

Although many marine species with restricted thermal tolerance reach their geographic limit at the Yangtze River, other species have distributions that span both sides of the river (Xu 1997). This raises the question of whether the CDW is a barrier to gene flow between populations of these species. In other marine realms, some well-known biogeographic boundaries, such as Point Conception in the eastern Pacific and Cape Hatteras on the mid-Atlantic coast of North America, have proven to be intraspecific barriers that restrict the gene flow of some wide-ranging taxa (Boehm *et al.* 2015; McCartney *et al.* 2013, but see Burton 1998), a phenomenon known as ‘biogeography-phylogeography concordance’ (Avice *et al.* 1987). Recently, many studies have tested whether the CDW is a phylogeographic barrier in addition to being a biogeographic boundary (Ni *et al.* 2014). However, the emerging pictures from these studies are not consistent, and controversy has arisen as different genetic patterns have appeared (e.g. Du *et al.* 2016; Liu *et al.* 2011; Yang *et al.* 2008).

A full understanding of the barrier effect of the CDW and the nature of the underlying mechanisms can only be properly attained by combining multiple lines of evidence from paleoceanography, topography, ecology and phylogeography (see Briggs & Bowen 2013; Burton 1998; Rocha *et al.* 2002). In this paper, we review evidence from various studies about the CDW to answer these questions. We first introduce the historical context of the Yangtze River outflow, and then address the entire known body of genetic work relating to its barrier effects. Those studies that document support for the barrier hypothesis are further examined for any common mechanisms, and a new hypothesis is proposed with support from a paleoceanographic perspective. Finally we offer several practical suggestions for future studies.

The Yangtze River outflow: history and features

The onset of the Yangtze River draining the Qinghai-Tibet Plateau and flowing into the ECS is dated at least 23 million years ago (Zheng *et al.* 2013). Eustatic sea level fluctuations through the late Quaternary might have dramatically changed the hydrological features of the paleo-Yangtze River outflow (Uehara & Saito 2003). However, due to changed or overlaid sediment records, the history of the paleo-Yangtze River mouth beyond 8 thousand years before the present (ka BP) is not well understood (Xiao *et al.* 2004). A widely accepted view is that during the last glacial maximum (LGM) when the sea level dropped more than 120 m, the paleo-river traversed the exposed sea shelf and persistently flowed into the Okinawa Trough, although the exact location of the flow channel and river mouth shifted (Chen *et al.* 2000; Uehara *et al.* 2002). An opposing opinion holds that prehistorically, the river flowed into an inland lake in northern China rather than entering the ECS, and that the river was once dry during the low sea-level period (Zhao 1984). After 8 ka BP, accompanied by marine transgression as the sea level rose, the present Yangtze River delta system began to develop,

and between 6 to 7 ka BP it prograded gradually southeast by building successive estuarine sand bars (Chen 1998; Hori *et al.* 2002; Liu *et al.* 1992; Xiao *et al.* 2004).

Today the CDW enters the ECS at latitude 31°N—the Yangtze Estuary (Fig. 1A), and causes significant environmental changes in the ECS. First, a strong salinity gradient surrounding the river mouth is generated by the collision of the CDW with several coastal currents (e.g. the Subei Coastal Current and the China Coastal Current) (Su & Yuan 2005) (Fig. 1B). In summer, with the significant increase in discharge, the low-salinity water plume can even extend northeastwards to the Korea/Tsushima Strait (Isobe *et al.* 2002), decreasing the salinity of the upper layer of the Kuroshio Current (Wu *et al.* 2010). Low salinity can have detrimental effects on marine species' larval behavior or development; for instance, when salinity is < 15 PSU, mantis shrimp (*Oratosquilla oratoria*) larvae stop feeding (Liu *et al.* 2012) and the development of surf clam (*Macra chinensis*) larvae is constrained (Liu *et al.* 2007c). Another significant environmental change arises from the huge Yangtze-derived mud deposit on the inner shelf (Liu *et al.* 2007a). Combined with contributions from other rivers, a large delta plain with an area of more than 3×10^4 km² has developed (Saito *et al.* 2001), and a sedimentary coast of mud substrate has been formed from Lianyungang (34°36'N, 119°13'E) to Shaoxing (30°19'N, 120° 46'E) with a straight-line distance of over 500 km (Zhu *et al.* 2008) (Fig. 1A). These environmental changes may have drastically influenced phylogeographic patterns of coastal species (with low tolerance to salinity changes or with habitat specificity) and left genetic signatures in contemporary populations (Wang *et al.* 2015; Xiao *et al.* 2016b).

Discordant phylogeographic patterns across the CDW

The barrier impact of the CDW on coastal species has been tested in various marine taxa,

et al. 2015; Ni *et al.* 2015b; Wang *et al.* 2015), crustaceans (e.g. Du *et al.* 2016; Han *et al.* 2015b) and macroalgae (Cheang *et al.* 2010). Based on multiple studies, it appears the resulting patterns are not universal, but rather species-specific: the phylogeographic break attributable to the CDW was evident in some species (e.g. Dong *et al.* 2012; Ni *et al.* 2015b; Wang *et al.* 2015), while not notable in some others (Liu *et al.* 2011; Ni *et al.* 2012; Xue *et al.* 2014; Yang *et al.* 2008). Below we explore reasons for this discordance in further detail.

Discordance among taxonomic groups

It is not surprising that discordant patterns have been observed among different taxonomic groups. In diverse evolutionary lineages, organisms usually show species-specific reproductive strategies, larval types (e.g. planktonic vs. direct development), planktonic larval durations (PLD) and habitat requirements (e.g. hard or soft substrate) that affect their responses to environmental factors (Ayre *et al.* 2009).

One common difference in response to the CDW has been identified between fishes and molluscs (reviewed in Ni *et al.* 2014). Fishes are generally less impacted by the CDW, since they can usually (though not always) maintain population connectivity over great distances because of their swimming abilities as adults and a relatively long PLD (e.g. da Silva *et al.* 2015; Gaither *et al.* 2010). A well-documented case in the ECS is the silver pomfret fish (*Pampus argenteus*). This species has a PLD of about 30 days, and shows substantial mitochondrial homogeneity across the CDW (Zhao *et al.* 2011). In comparison, molluscan species, which usually have a sessile life history and occupy a narrow strip of coastal range (one dimension), are more prone to be affected by biogeographic and historical barriers (Kelly & Palumbi 2010; Sagarin & Gaines 2002). Examples in the ECS include two *Octopus* species, which lack a planktonic larval stage and show significant genetic breaks between neighboring populations (Chang *et al.* 2010; Lü *et al.* 2010). Indeed, a phylogeographic

review of studied marine species of the ECS has demonstrated that 88.9% of molluscan species have significant population structure while for fishes the proportion is much lower, about 37.5% (Ni *et al.* 2014). However, in practice, pinpointing the specific factor that renders different patterns among evolutionarily diverse taxa is not straightforward (Dawson 2012). Multiple environmental variables (e.g., temperature, salinity, ocean currents and geography) may act in combination with stochastic processes such as local selection and lineage sorting through time (Miglietta *et al.* 2011; Palumbi 1994).

Discordance among congeners

Besides discordance among evolutionarily distant species, different phylogeographic patterns have also been discovered between congeneric species with similar life histories. For example, *M. chinensis* and *M. veneriformis* are two common surf clams in the ECS with a PLD of about 10 days and are found in sand or muddy sand bottom burrows from low-tide zones to shallow waters (Qi 2004; Wang & Wang 2008; Wang *et al.* 1984). Despite their similar habitat preferences and life histories, genetic studies revealed discordant phylogeographic patterns between them: *M. veneriformis* was genetically homogeneous in the ECS with the central and other internal haplotypes widely shared among all populations (Ni *et al.* 2015a) (Fig. 2A), while *M. chinensis* showed significant population subdivision across the CDW with no haplotypes shared between populations on either side of the river (Ni *et al.* 2015b) (Fig. 2B). The lack of stepping-stone populations for *M. chinensis* across the river mouth is thought to be responsible for this subdivision (Xu 1997). Alternatively, the southern population might be more susceptible to loss and reorganization of variation by genetic drift because of its isolation from the northern populations.

Because phylogeography is not known for enough congeneric species in the ECS, it is still unclear why and how frequently discordant patterns arise among closely related species in the

region. Further research is warranted to provide a more comprehensive view. Worldwide, starkly contrasting biogeographic patterns between congeneric species have been revealed in studies of marine bivalves (Marko & Moran 2009), gastropods (Crandall *et al.* 2008; Marko 2004), seahorses (Lourie *et al.* 2005) and butterflyfishes (DiBattista *et al.* 2012). A broad range of factors, such as differences in life-history, ecology and habitat specificity, has been interpreted as driving forces in this discordance (see Ayre *et al.* 2009; Dawson *et al.* 2002; Marko 2004 for detailed information).

Studies claiming the CDW is a barrier: all that glitters is not gold

For phylogeographic studies that conclude the CDW is a physical barrier, it is still unclear whether genetic divergence in the studied species is similar in magnitude (in terms of time scale) and whether they have been caused by the same factors (e.g., salinity, temperature, habitat discontinuity or a combination thereof). Here we provide a comprehensive overview of these questions by synthesizing studies that claim the CDW is a marine phylogeographic barrier. Literature searches (until April 2017) were conducted in both Web of Science and China Integrated Knowledge Resources Database (<http://www.cnki.net/>), and further complemented by searches in Google Scholar (<https://scholar.google.com/>). Different key phrases and combinations (e.g. ‘Changjiang Diluted Water’, ‘Yangtze River outflow’, ‘marine barrier’, ‘genetic structure’ and ‘phylogeography’) were used to ensure extensive coverage. In total, 16 phylogeographic studies (on 13 species and one genus) that conformed to our research scope were retrieved (see Table 1). After carefully reading and rechecking the studies’ results, we found that although all these studies concluded that the CDW had acted as a phylogeographic barrier, the reported genetic patterns in fact did not always agree with the proposed underlying mechanisms. We divide the studies into three categories (Table 1) according to taxonomic level and genetic patterns, and discuss the potential impact of the

CDW on each category below.

Category I. The CDW as a biogeographic barrier

The first category covers studies that confuse the concept of biogeographic barrier with phylogeographic barrier. Examples include two studies on the macroalga *Sargassum hemiphyllum* (Cheang *et al.* 2010) and the limpet genus *Nipponacmea* along coastal China (Yu *et al.* 2014). The CDW was demonstrated to have restricted the northern distribution of *Sargassum hemiphyllum var. chinense* and the cross-estuary distribution of the *Nipponacmea* species, respectively. In a strict sense, in these studies, the CDW should be defined as a biogeographic barrier rather than a phylogeographic barrier because it acts on the species level. As mentioned in the ‘Introduction’, the CDW represents a biogeographic boundary between cold temperate and tropical/subtropical faunas of the northwestern Pacific, and restricts the distribution of many marine species to one biogeographic region (for example, *N. radula* to the north and *N. nigrans* to the south of the CDW, as shown in Fig. 3A).

Category II. Deep divergence mistakenly attributed to the CDW

Category II includes five published studies on three mollusc and one crustacean species: *Coelomactra antiquata* (Kong *et al.* 2007), *Cyclina sinensis* (Zhao *et al.* 2007; Zhao *et al.* 2009), *Octopus ocellatus* (Lü *et al.* 2010) and *Fistulobalanus albicostatus* (Chang *et al.* 2017). Each study documented deep intraspecific divergence with spatial trends consistent with allopatric separation arising from the CDW. However, great caution is needed when interpreting the mechanism behind this divergence. For instance, for *C. sinensis*, the role of the CDW as a phylogeographic barrier in Zhao *et al.* (2007, 2009) was rejected by a further appraisal that utilized more comprehensive sampling and multiple genetic data sets (Ni *et al.* 2012). The divergence in fact arose from the historical isolation of populations in sea basins

followed by postglacial dispersal, rather than being caused by the CDW (see Ni *et al.* 2012, and Fig. 2, 3 therein). Studies in this category are at risk of mistakenly attributing deep genetic divergence to the CDW. This misunderstanding may be caused by insufficient sampling coverage, especially when populations from the vicinity of the Yangtze estuary are lacking. The same problem may exist in the study of *C. antiquate* (Kong *et al.*, 2007), but further analyses using two independent molecular markers (AFLP and mitochondrial 16S rDNA) did not reach a compelling conclusion due to a lack of intermediate populations (Kong & Li, 2009; Fig. 3B).

Category III. Shallow genetic divergence likely resulting from the CDW

Phylogeographic patterns documented in the nine studies that make up Category III uniformly revealed shallow haplotype networks, with a ‘star-like’ topology in most cases. However, in these studies uneven spatial distribution of certain haplotypes across the CDW is observed, resulting in significant population structure between populations on the two sides (e.g. Dong *et al.* 2012; Ni *et al.* 2015b). An example is shown in Fig. 3C, where the haplotype H2 of *Cellana toreuma* (one mutation apart from the central haplotype H1) was abundant in two northern populations of the CDW, but not observed in southern populations. Based on this distributional trend, the CDW is believed to be the most likely factor limiting the southward dispersal of larvae from northern region (Dong *et al.* 2012). Similarly, in the other 8 cases of category III, the CDW is presumed to have acted as a contemporary barrier that limits the distribution of the newly derived haplotypes, and is responsible for the observed population structure. Larval tolerance to salinity (e.g. Han *et al.* 2015b) and habitat discontinuity across the estuary (e.g. Dong *et al.* 2016; Dong *et al.* 2012), and sometimes the combined effects of these two factors (Ni *et al.* 2015b) are thought to be the major influential mechanisms.

A paleoceanographic perspective on the ECS

Paleoceanographic features of the ECS support the conclusion that the CDW has not been a historically persistent barrier for coastal species in this region. The ECS, including the Bohai Gulf and the Yellow Sea (usually treated as a single contiguous sea in phylogeographic studies), is one of the largest marginal seas in the world (Wang 1999) (Fig. 1A). Surrounded by mainland China, Taiwan Island, the Korean Peninsula, Kyushu Island and the Ryukyu Arc, the ECS continental shelf covers a total area of $8.5 \times 10^5 \text{ km}^2$ (Xu & Oda 1999). Dramatic climatic changes have occurred in the ECS since its formation in the late Cenozoic (Wang 1999), including eustatic sea level fluctuations and successive exposure and inundation of the shelf. When glaciations advanced, the sea level of the ECS declined more than 120 m below its present level, and the sea retreated to an elongated and curved basin—the Okinawa Trough (Xie *et al.* 1995). The Okinawa Trough was spatially separated from the South China Sea by a large land bridge extending between eastern China and Taiwan Island (Kimura 2000) (Fig. 1A).

The present-day marine species whose ranges span the CDW may have different colonization histories: some might have become established in the ECS before the LGM and persisted in the region through multiple Pleistocene glacial-interglacial cycles, while others might have appeared relatively recently, having only successfully colonized the ECS after the LGM. In the case of species that became established before the LGM, their distribution might have experienced significant and repetitive expansion and contraction through successive glacio-eustatic cycles (McManus 1985). When the glaciers advanced, the loss of suitable habitat would have forced survivors into the Okinawa refugium, which has an area less than 1/3 of the present size of the ECS (Xu *et al.* 2009). Thus geographically distant, isolated populations would have mixed together to form an ancestral panmictic population in the

Okinawa Trough (Liu *et al.* 2011). Therefore, even if the paleo-Yangtze River had impacted the population subdivision of these historically persistent species, any genetic signals would have been swamped by shifting river channel locations and repeated sea level fluctuations (Liu *et al.* 2011).

Thus, we may conclude that the CDW has not been a stable and persistent barrier capable of causing deep divergence between populations, regardless of species' residence time in the ECS. Spatial isolation of populations resulting from the CDW can only be dated back to the LGM, about 15 ka BP (Xiao *et al.* 2004). This time scale is hardly comparable to isolation time resulting from the long-standing separation of sea basins (on the scale of millions of years) (Liu *et al.* 2011; Zheng *et al.* 2009).

Suggestions for future studies

The CDW is not the only driver promoting genetic break in the northwestern Pacific. Other historical or contemporary factors have also been proven to act on population differentiation, including ocean currents (Tsang *et al.* 2008), sea-level oscillations coupled with Pleistocene glaciations (Liu *et al.* 2007b), and temperature gradients (Dong *et al.* 2015; Ni *et al.* 2015b; Shen *et al.* 2011). Future phylogeographic studies in this region should be carefully designed (e.g., taking into account adequate sample size, geographic coverage, and appropriate choice of biomarkers) to ensure that different competing hypotheses are adequately tested. Here we provide some suggestions for future studies.

First, population sampling should be emphasized in adjacent regions of the Yangtze Estuary, approximately from Lianyungang to Xiamen (see Fig. 1A). Intensive sampling in this region can help pinpoint the specific evolutionary scenario behind any observed genetic breaks, and better identify the location of physical barrier. For example, in a study of the Asian paddle crab *Charybdis japonica*, intensive sampling of populations around

Lianyungang allowed Han *et al.* (2015a) to identify the Haizhou Bay (the coastal waters of Lianyungang) as a physical barrier rather than the Yangtze Estuary.

Second, considering that the CDW has likely affected population structure more recently than previously believed, it is advisable to use (or combine) independent nuclear markers with high resolution, such as microsatellites and single nucleotide polymorphisms (SNPs). Former studies heavily rely on a single mitochondrial marker, a scheme that has been criticized for well-known reasons including limited resolution power and incomplete capture of evolutionary history (see details in Edwards & Bensch 2009, and Galtier *et al.* 2009). Recent applications of next generation sequencing methods such as restriction-site associated DNA sequencing have proven very powerful for identifying fine-scale phylogeographic patterns (e.g. Edwards *et al.* 2015; Emerson *et al.* 2010; Reitzel *et al.* 2013). These up-to-date approaches hold much promise for providing a more in-depth understanding of the CDW's barrier effect.

Third, mounting genetic evidence shows that a barrier causing population partitioning in one species does not always cause partitioning in other species—even between congeneric species with similar life histories (e.g. Crandall *et al.* 2008; DiBattista *et al.* 2012). For the CDW issue, this implies that more comparative phylogeographic studies using additional species pairs are needed to explain underlying mechanism rendering these types of discrepancies. Species from diverse taxonomic groups such as Cnidaria, Annelida and Echinodermata represent good candidates for such comparative studies as they are abundant in this region (Liu 2013), and suffer less from human-mediated activities compared with commercially exploited fishes and molluscs (Ni *et al.* 2014).

Conclusions

Based on multiple lines of evidences from paleoceanography, ecology and phylogeography, this study sheds light on the phylogeographic question of whether and how the CDW acts as a barrier. The answer to this, however, is not a simple yes-or-no: the conclusion may depend on the species selected for study. This is consistent with the intrinsic nature of ‘soft’ barriers in the ocean (i.e. barriers involved with hydrological processes, e.g. water currents), which act as dispersal ‘filters’ that restrict selected species as opposed to physical obstacles such as landmasses that are impassable to all animal forms (Luiz *et al.* 2012; Rocha *et al.* 2002). Although the CDW is an influential environmental factor for both historical and contemporary populations, its barrier effect is best assumed to have acted on a recent time scale (presumably after the LGM). Still, considering the uncertainty about the paleogeographic history of the CDW and tenuo forms

References

- Avise JC, Arnold J, Ball RM, *et al.* (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489-522.
- Ayre DJ, Minchinton TE, Perrin C (2009) Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? *Molecular Ecology*, **18**, 1887-1903.
- Beardsley RC, Limeburner R, Yu H, Cannon GA (1985) Discharge of the Changjiang (Yangtze river) into the East China sea. *Continental Shelf Research*, **4**, 57-76.
- Boehm J, Waldman J, Robinson JD, Hickerson MJ (2015) Population genomics reveals seahorses (*Hippocampus erectus*) of the western mid-Atlantic Coast to be residents rather than vagrants. *PLoS ONE*, **10**, e0116219.
- Briggs JC, Bowen BW (2013) Marine shelf habitat: biogeography and evolution. *Journal of Biogeography*, **40**, 1023-1035.
- Burton RS (1998) Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution*, **52**, 734-745.
- Chang KM, Li H, Lü ZM, Chi CF (2010) Genetic variation in different populations of *Octopus variabilis* in China coastal waters based on the COI gene analysis. *Oceanologia et Limnologia Sinica*, **41**, 307-314.
- Chang YW, Chan JS, Hayashi R, *et al.* (2017) Genetic differentiation of the soft shore barnacle *Fistulobalanus albicostatus* (Cirripedia: Thoracica: Balanomorpha) in the West Pacific. *Marine Ecology*, **38**, e12422.
- Cheang CC, Chu KH, Ang Jr PO (2010) Phylogeography of the marine macroalga *Sargassum hemiphyllum* (Phaeophyceae, Heterokontophyta) in northwestern Pacific. *Molecular Ecology*, **19**, 2933-2948.
- Chen XQ (1998) Changjiang (Yangtze) River Delta, China. *Journal of Coastal Research*, **14**, 839-858.
- Chen ZY, Song BP, Wang ZH, Cai YL (2000) Late Quaternary evolution of the sub-aqueous Yangtze Delta, China: sedimentation, stratigraphy, palynology, and deformation. *Marine Geology*, **162**, 423-441.
- Condie SA, Mansbridge JV, Cahill ML (2011) Contrasting local retention and cross-shore transports of the East Australian Current and the Leeuwin Current and their relative influences on the life histories of small pelagic fishes. *Deep Sea Research Part II: Topical Studies in Oceanography*, **58**, 606-615.
- Crandall ED, Frey MA, Grosberg RK, Barber PH (2008) Contrasting demographic history and phylogeographical patterns in two Indo-Pacific gastropods. *Molecular Ecology*, **17**, 611-626.
- Da Silva R, Veneza I, Sampaio I, *et al.* (2015) High levels of genetic connectivity among populations of yellowtail snapper, *Ocyurus chrysurus* (Lutjanidae–Perciformes), in the western South Atlantic revealed

through multilocus analysis. *PLoS ONE*, **10**, e0122173.

Dawson MN (2012) Parallel phylogeographic structure in ecologically similar sympatric sister taxa. *Molecular Ecology*, **21**, 987-1004.

Dawson MN, Louie KD, Barlow M, Jacobs DK, Swift CC (2002) Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. *Molecular Ecology*, **11**, 1065-1075.

De Bruyn M, Nugroho E, Hossain MM, Wilson J, Mather P (2005) Phylogeographic evidence for the existence of an ancient biogeographic barrier: the Isthmus of Kra Seaway. *Heredity*, **94**, 370-378.

DiBattista JD, Rocha LA, Craig MT, Feldheim KA, Bowen BW (2012) Phylogeography of two closely related Indo-Pacific butterflyfishes reveals divergent evolutionary histories and discordant results from mtDNA and microsatellites. *Journal of Heredity*, **103**, 617-629.

Dong YW, Han GD, Ganmanee M, Wang J (2015) Latitudinal variability of physiological responses to heat stress of the intertidal limpet *Cellana toreuma* along the Asian coast. *Marine Ecology Progress Series*, **529**, 107-119.

Dong YW, Huang XW, Wang W, Li Y, Wang J (2016) The marine 'great wall' of China: local- and broad- scale ecological impacts of coastal infrastructure on intertidal macrobenthic communities. *Diversity and Distributions*, **22**, 731-744.

Dong YW, Wang HS, Han GD, *et al.* (2012) The impact of Yangtze river discharge, ocean currents and historical events on the biogeographic pattern of *Cellana toreuma* along the China coast. *PLoS ONE*, **7**, e36178.

Du XW, Cai SS, Yu CG, *et al.* (2016) Population genetic structure of mantis shrimps *Oratosquilla oratoria*: Testing the barrier effect of the Yangtze River outflow. *Biochemical Systematics and Ecology*, **66**, 12-18.

Edwards SV, Bensch S (2009) Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. *Molecular Ecology*, **18**, 2930-2933.

Edwards SV, Shultz AJ, Campbell-Staton SC (2015) Next-generation sequencing and the expanding domain of phylogeography. *Folia Zoologica*, **64**, 187-206.

Emerson KJ, Merz CR, Catchen JM, *et al.* (2010) Resolving postglacial phylogeography using high-throughput sequencing. *Proceedings of the National Academy of Sciences*, **107**, 16196-16200.

Gaither MR, Toonen RJ, Robertson DR, Planes S, Bowen BW (2010) Genetic evaluation of marine biogeographical barriers: perspectives from two widespread Indo-Pacific snappers (*Lutjanus kasmira* and *Lutjanus fulvus*). *Journal of Biogeography*, **37**, 133-147.

- Galtier N, Nabholz B, Glémin S, Hurst GDD (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology*, **18**, 4541-4550.
- Gong GC, Chen YLL, Liu KK (1996) Chemical hydrography and chlorophyll a distribution in the East China Sea in summer: implications in nutrient dynamics. *Continental Shelf Research*, **16**, 1561-1590.
- Guo X, Zhao D, Jung D, *et al.* (2015) Phylogeography of the rock shell *Thais clavigera* (Mollusca): Evidence for long-distance dispersal in the Northwestern Pacific. *PLoS ONE*, **10**, e0129715.
- Han ZQ, Zheng W, Zhu WB, *et al.* (2015a) A barrier to gene flow in the Asian paddle crab, *Charybdis japonica*, in the Yellow Sea. *ICES Journal of Marine Science*, **72**, 1440-1448.
- Han ZQ, Zhu WB, Zheng W, Li PF, Shui BN (2015b) Significant genetic differentiation between the Yellow Sea and East China Sea populations of cocktail shrimp *Trachypenaeus curvirostris* revealed by the mitochondrial DNA COI gene. *Biochemical Systematics and Ecology*, **59**, 78-84.
- Holt BG, Lessard J-P, Borregaard MK, *et al.* (2013) An update of Wallace's zoogeographic regions of the world. *Science*, **339**, 74-78.
- Hori K, Saito Y, Zhao QH, Wang PX (2002) Architecture and evolution of the tide-dominated Changjiang (Yangtze) River delta, China. *Sedimentary Geology*, **146**, 249-264.
- Isobe A, Ando M, Watanabe T, *et al.* (2002) Freshwater and temperature transports through the Tsushima-Korea Straits. *Journal of Geophysical Research: Oceans*, **107**, C7, 3065.
- Johnson DR, Boyer TP (2015) East Asian Seas Regional Climatology (Version 2). National Centers for Environmental Information, NOAA, USA, dataset.
- Kelly RP, Palumbi SR (2010) Genetic structure among 50 species of the northeastern Pacific rocky intertidal community. *PLoS ONE*, **5**, e8594.
- Kendrick GA, Walker DI (1995) Dispersal of propagules of *Sargassum spp.* (Sargassaceae: Phaeophyta): Observations of local patterns of dispersal and consequences for recruitment and population structure. *Journal of Experimental Marine Biology and Ecology*, **192**, 273-288.
- Kimura M (2000) Paleogeography of the Ryukyu Islands. *Tropics*, **10**, 5-24.
- Kirkendale LA, Meyer CP (2004) Phylogeography of the Patelloida profunda group (Gastropoda: Lottidae): diversification in a dispersal-driven marine system. *Molecular Ecology*, **13**, 2749-2762.
- Kong LF, Li Q (2009) Genetic evidence for the existence of cryptic species in an endangered clam *Coelomastra antiquata*. *Marine Biology*, **156**, 1507-1515.
- Kong LF, Li Q, Qiu ZX (2007) Genetic and morphological differentiation in the clam *Coelomastra antiquata*

(Bivalvia: Veneroida) along the coast of China. *Journal of Experimental Marine Biology and Ecology*, **343**, 110-117.

Liu H, He Q, Wang ZB, Weltje GJ, Zhang J (2010) Dynamics and spatial variability of near-bottom sediment exchange in the Yangtze Estuary, China. *Estuarine, Coastal and Shelf Science*, **86**, 322-330.

Liu HY, Wang DX, Jiang YS, Chen L (2012) The effects of salinity on survival and food intake in mantis shrimp *Oratosquilla oratoria* larvae. *Journal of Dalian Ocean University*, **27**, 311-314.

Liu JY (2013) Status of marine biodiversity of the China Seas. *PLoS ONE*, **8**, e50719.

Liu J, Li Q, Kong LF, Zheng XD (2011) Cryptic diversity in the pen shell *Atrina pectinata* (Bivalvia: Pinnidae): high divergence and hybridization revealed by molecular and morphological data. *Molecular Ecology*, **20**, 4332-4345.

Liu JP, Xu KH, Li AC, *et al.* (2007a) Flux and fate of Yangtze River sediment delivered to the East China Sea. *Geomorphology*, **85**, 208-224.

- McCartney MA, Burton ML, Lima TG (2013) Mitochondrial DNA differentiation between populations of black sea bass (*Centropristis striata*) across Cape Hatteras, North Carolina (USA). *Journal of Biogeography*, **40**, 1386-1398.
- McManus J (1985) Marine speciation, tectonics and sea-level changes in southeast Asia. *Proceedings of the Fifth International Coral Reef Congress, Tahiti*, **4**, 133-138.
- Miglietta MP, Faucci A, Santini F (2011) Speciation in the sea: overview of the symposium and discussion of future directions. *Integrative and Comparative Biology*, **51**, 449-455.
- Ni G, Li Q, Kong LF, Yu H (2014) Comparative phylogeography in marginal seas of the northwestern Pacific. *Molecular Ecology*, **23**, 534-548.
- Ni G, Li Q, Kong LF, Yu H (2015a) Mitochondrial phylogeography of a surf clam *Mactra veneriformis* in the East China Sea: Genetic homogeneity across two biogeographic boundaries. *Biochemical Systematics and Ecology*, **61**, 493-500.
- Ni G, Li Q, Kong LF, Zheng XD (2012) Phylogeography of bivalve *Cyclina sinensis*: testing the historical glaciations and Changjiang River outflow hypotheses in northwestern Pacific. *PLoS ONE*, **7**, e49487.
- Ni G, Li Q, Ni LH, Kong LF, Yu H (2015b) Population subdivision of the surf clam *Mactra chinensis* in the East China Sea: Changjiang River outflow is not the sole driver. *PeerJ*, **3**, e1240.
- Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, **25**, 547-572.
- Qi ZY (2004) *Seashells of China*. China Ocean Press, Beijing, China.
- Reitzel AM, Herrera S, Layden M, Martindale M, Shank T (2013) Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. *Molecular Ecology*, **22**, 2953-2970.
- Rocha LA, Bass AL, Robertson DR, Bowen BW (2002) Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). *Molecular Ecology*, **11**, 243-252.
- Sagarin RD, Gaines SD (2002) Geographical abundance distributions of coastal invertebrates: Using one-dimensional ranges to test biogeographic hypotheses. *Journal of Biogeography*, **29**, 985-997.
- Saito Y, Yang ZS, Hori K (2001) The Huanghe (Yellow River) and Changjiang (Yangtze River) deltas: a review on their characteristics, evolution and sediment discharge during the Holocene. *Geomorphology*, **41**, 219-231.
- Schlitzer R, Ocean Data View, <http://odv.awi.de>, 2015.

- Shen KN, Jamandre BW, Hsu CC, Tzeng WN, Durand JD (2011) Plio-Pleistocene sea level and temperature fluctuations in the northwestern Pacific promoted speciation in the globally-distributed flathead mullet *Mugil cephalus*. *BMC Evolutionary Biology*, **11**, 83.
- Su JL, Yuan YL (2005) *Coastal Hydrology of China*. Ocean Press, Beijing, China.
- Taylor MS, Hellberg ME (2006) Comparative phylogeography in a genus of coral reef fishes: biogeographic and genetic concordance in the Caribbean. *Molecular Ecology*, **15**, 695-707.
- Tsang LM, Chan BKK, Ma KY, Chu KH (2008) Genetic differentiation, hybridization and adaptive divergence in two subspecies of the acorn barnacle *Tetraclita japonica* in the northwestern Pacific. *Molecular Ecology*, **17**, 4151-4163.
- Uehara K, Saito Y (2003) Late Quaternary evolution of the Yellow/East China Sea tidal regime and its impacts on sediments dispersal and seafloor morphology. *Sedimentary Geology*, **162**, 25-38.
- Uehara K, Saito Y, Hori K (2002) Paleotidal regime in the Changjiang (Yangtze) Estuary, the East China Sea, and the Yellow Sea at 6 ka and 10 ka estimated from a numerical model. *Marine Geology*, **183**, 179-192.
- Wang BD, Wang XL, Zhan R (2003) Nutrient conditions in the Yellow Sea and the East China Sea. *Estuarine, Coastal and Shelf Science*, **58**, 127-136.
- Wang J, Tsang LM, Dong YW (2015) Causations of phylogeographic barrier of some rocky shore species along the Chinese coastline. *BMC Evolutionary Biology*, **15**, 114.
- Wang PX (1999) Response of western Pacific marginal seas to glacial cycles: paleoceanographic and sedimentological features. *Marine Geology*, **156**, 5-39.
- Wang RC, Wang ZP (2008) *Science of marine shellfish culture*. China Ocean University Press, Qingdao, China.
- Wang ZC, Liu JM, Zhu A, Li WJ, Shen YC (1984) A preliminary study on the biology of the Chinese surf clam (*Macra chinensis*) in Yalu River Estuary. *Journal of Fisheries of China*, **8**, 33-44.
- Wu ZY, Chen HX, Liu N (2010) Relationship between east China Sea Kuroshio and climatic elements in East China. *Marine Science Bulletin*, **12**, 1-9.
- Xiao SB, Li AC, Jiang FQ, *et al.* (2004) The history of the Yangtze River entering sea since the Last Glacial Maximum: a review and look forward. *Journal of Coastal Research*, **20**, 599-604.
- Xiao YS, Li J, Ren GJ, *et al.* (2016a) Pronounced population genetic differentiation in the rock bream *Oplegnathus fasciatus* inferred from mitochondrial DNA sequences. *Mitochondrial DNA*, **27**, 2045-2052.
- Xiao YS, Ma DY, Dai M, *et al.* (2016b) The impact of Yangtze River discharge on the genetic structure of a population of the rock bream, *Oplegnathus fasciatus*. *Marine Biology Research*, **12**, 426-434.

- Xie C, Jian Z, Zhao Q (1995) Paleogeographic maps of the China Seas at the last glacial maximum. In: *WESTPAC Paleogeographic Maps*, p. 75. UNESCO/IOC Publications, Shanghai.
- Xu FS (1997) *Bivalve Mollusca of China Seas*. Science Press, Beijing, China.
- Xu J, Chan TY, Tsang LM, Chu KH (2009) Phylogeography of the mitten crab *Eriocheir sensu stricto* in East Asia: Pleistocene isolation, population expansion and secondary contact. *Molecular Phylogenetics and Evolution*, **52**, 45-56.
- Xu SY, Song N, Lu ZC, *et al.* (2014) Genetic variation in scaly hair-fin anchovy *Setipinna tenuifilis* (Engraulididae) based on the mitochondrial DNA control region. *Mitochondrial DNA*, **25**, 223-230.
- Xu X, Oda M (1999) Surface-water evolution of the eastern East China Sea during the last 36,000 years. *Marine Geology*, **156**, 285-304.
- Xue DX, Wang HY, Zhang T, Liu JX (2014) Population genetic structure and demographic history of *Atrina pectinata* based on mitochondrial DNA and microsatellite markers. *PLoS ONE*, **9**, e95436.
- Yang JM, Li Q, Zheng XD, Song ZL, Wang RC (2008) Genetic diversity in populations of *Rapana venosa* in coastal waters of China. *Oceanologia et Limnologia Sinica*, **39**, 257-262.
- Yu SS, Wang J, Wang QL, Huang XW, Dong YW (2014) DNA barcoding and phylogeographic analysis of *Nipponacmea* limpets (Gastropoda: Lottiidae) in China. *Journal of Molluscan Studies*, **80**, 420-429.
- Zhao F, Zhuang P, Zhang LZ, Shi ZH (2011) Population genetic structure of *Pampus argenteus* in the south Yellow Sea and East China Sea based on the mitochondrial *cyt b* sequence. *Acta Hydrobiologica Sinica*, **35**, 745-752.
- Zhao SL (1984) Quaternary geological questions of the Yangtze delta. *Marine sciences*, **5**, 15-20.
- Zhao YM, Li Q, Kong LF, Bao ZM, Wang RC (2007) Genetic diversity and divergence among clam *Cyclina sinensis* populations assessed using amplified fragment length polymorphism. *Fisheries Science*, **73**, 1338-1343.
- Zhao YM, Li Q, Kong LF, Mao Y (2009) Genetic and morphological variation in the venus clam *Cyclina sinensis* along the coast of China. *Hydrobiologia*, **635**, 227-235.
- Zheng HB, Clift PD, Wang P, *et al.* (2013) Pre-Miocene birth of the Yangtze River. *Proceedings of the National Academy of Sciences*, **110**, 7556-7561.
- Zheng WJ, Zhu SH, Shen XQ, *et al.* (2009) Genetic differentiation of *Tegillarca granosa* based on mitochondrial COI gene sequences. *Zoological Research*, **30**, 17-23.

Zhu ZW, Gao MS, Zhu YF (2008) Quantitative analysis of basic types of coastal zones and their distribution. *Earth Science Frontiers*, 7, 24-28.

Figure legends

Fig. 1 Map of East Asia showing seafloor topography (based on Schlitzer 2015) and ecological factors (based on Johnson & Boyer 2015). (A) The Yangtze River and the marginal seas of the northwestern Pacific. The dotted line separates two biogeographic regions: I- North Pacific Temperate Biotic Region and II- Indo-West Pacific Warm-water Biotic Region. Four locations mentioned in the study are also marked. (B) annual salinity (in practical salinity units, PSU) and (C) annual temperature (°C) at the surface (ten-degree grid). *SBCC*: Subei Coastal Current; *CCC*: China Coastal Current.

Fig. 2 Sampling locations and haplotype relationship of the surf clam (A) *Macraa veneriformis* (redrawn based on Ni *et al.* 2015a) and (B) *M. chinensis* (Ni *et al.* 2015b). The sizes of the circles are proportional to haplotype frequencies in each study, and mutation steps between haplotypes are illustrated using bars. Populations north of the Yangtze River are shown in red while the southern ones are in blue.

Fig. 3 Representative cases for three different categories of studies of the CDW summarized in Table 1. (A) The CDW as a biogeographic barrier for *Nipponacmea* (redrawn based on Yu *et al.* 2014); (B) Deep genetic divergence in *Coelomacra antiquata*

Table 1 List of species used to test the phylogeographic barrier effect of the Yangtze River outflow. Category I: the CDW as a biogeographic barrier; category II: deep divergence mistakenly attributed to the CDW; category III: shallow genetic divergence likely resulting from the CDW.

Species	Group	Habitat and distribution	Larval type and duration	Markers and populations	Major conclusion	Reference
Category I						
<i>Nipponacmea</i> (genus)	Mollusc	Intertidal rocky shores of the western Pacific	Pelagic larvae. Duration time is unknown but 7-10 days is reported for limpets (Kirkendale & Meyer 2004)	COI, 28S rDNA and H3; Five northern populations of <i>N. radula</i> , and nine southern populations of <i>N. fuscoviridis</i> and <i>N. nigrans</i>	The Yangtze River is a phylogeographic barrier to the distribution of <i>Nipponacmea</i> along coastal China.	Yu <i>et al.</i> (2014)
<i>Sargassum hemiphyllum</i>	Alga	Low intertidal zone of Japan, Korea, China and Vietnam	Releases algal propagules (gametes, spores, zygotes) that settle within meters of their sources (Kendrick & Walker 1995)	ITS2, plastidial Rubisco (Rbc) and mitochondrial TrnW_I (Trn) spacers; Four southern populations and five northern ones	The Yangtze is a contemporary dispersal barrier between <i>Sargassum hemiphyllum</i> and <i>var. Chinese</i> .	Cheang <i>et al.</i> (2010)
Category II						
<i>Coelomacra antiquate</i>	Mollusc	Sandy habitats from the low intertidal zone to 20 m depth. Endemic in the NW Pacific: Japan, China	Pelagic larvae, 9-14 days	Seven allozyme loci and 12 morphological variables; three northern populations and one southern population	Differentiation between northern and southern populations. The Yangtze River is a barrier to gene flow.	Kong <i>et al.</i> (2007)
<i>Cyclina sinensis</i>	Mollusc	Widely distributed in muddy sand beaches in the intertidal zone to estuaries of East Asia	Pelagic larvae, 6-9 days	(1) AFLP; Two northern and two southern populations; (2) Allozyme loci and morphological variables; three northern and three southern populations	(1) (2) Differentiation between northern and southern populations because of isolation by the Yangtze River outflow.	(1) Zhao <i>et al.</i> (2007) (2) Zhao <i>et al.</i> (2009)

Octopus ocellatus

Mollusc

Coastal waters and
estuaries of China and

<i>Siphonaria japonica</i>	Mollusc	Intertidal rocky shores of Japan, Korea, China and Vietnam.	Egg ribbons and pelagic larvae, > 7 days	COI and ITS; Four northern populations and five southern ones	Significant population structure in line with the Yangtze River.	Wang <i>et al.</i> (2015)
<i>Oratosquilla oratoria</i>	Crustacean	Widely distributed along the northwestern Pacific marginal seas	Pelagic larvae, 36-54 days	16S rDNA; Four northern populations and three southern ones	Shallow divergence in line with the CDW.	Du <i>et al.</i> (2016)
<i>Macra chinensis</i>	Mollusc	Mainly distributed along the northern Chinese coastline, but with an isolated population in the south	Pelagic larvae, ~10 days	Seven northern populations and one southern population; COI and nine polymorphism microsatellite loci	One lineage with star-like topology; population subdivision was detected across the Yangtze River.	Ni <i>et al.</i> (2015)
<i>Oplegnathus fasciatus</i>	Fish	Widely distributed in rocky reefs of the southern part of Korea, Japan, China, and Hawaii.	Pelagic larvae, ~30 days	(1) COI, Cytb, D-loop; one northern population and two southern ones; (2) Ten microsatellite loci; one northern population and two southern ones	(1) Three shallow divergent clades; (2) Population differentiation across the Yangtze River.	(1) Xiao <i>et al.</i> (2016a) (2) Xiao <i>et al.</i> (2016b)

ITS: internal transcribed spacers; rDNA: ribosomal DNA; AFLP: amplified fragment length polymorphism; COI: cytochrome oxidase *I*; Cytb: cytochrome B; CR, control region; 12S: 12S ribosomal RNA.



