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ORIGINAL PAPER

Millennium-old farm breeding of Chinese softshell turtles (*Pelodiscus* spp.) results in massive erosion of biodiversity

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Abstract

Chinese softshell turtles (*Pelodiscus* spp.) are widely distributed, ranging from the Amur and Ussuri Rivers in the Russian Far East through the Korean Peninsula, Japan, and eastern, central, and southern China to southern Vietnam. In East and Southeast Asia, Chinese softshell turtles are traditionally exploited for food and have been farm-bred in China since the Spring and Autumn Period, more than 2400 years ago. Currently, the annual production of *Pelodiscus* amounts to 340,000 t in China alone. Using mitochondrial DNA (2428 bp) and five nuclear loci (3704 bp), we examined broad sampling of wild and farm-bred *Pelodiscus* to infer genetic and taxonomic differentiation. We discovered four previously unknown mitochondrial lineages, all from China. One lineage from Jiangxi is deeply divergent and sister to the mitochondrial lineage of *Pelodiscus axenaria*. The nuclear loci supported species status for *P. axenaria* and the new lineage from Jiangxi. *Pelodiscus maackii* and *P. parviformis*, both harboring distinct mitochondrial lineages, were not differentiated from *P. sinensis* in the studied nuclear markers. The same is true for two new mitochondrial lineages from Zhejiang, China, represented by only one individual each, and another new lineage from Anhui, Guangdong, Jiangxi and Zhejiang, China. However, Vietnamese turtles yielding a mitochondrial lineage clustering within *P. sinensis* were distinct in nuclear markers, suggesting that these populations could represent another unknown species with introgressed mitochondria. Its species status is also supported by the syntopic occurrence with *P. sinensis* in northern Vietnam and by morphology. In addition, we confirmed sympatry of *P. axenaria* and *P. parviformis* in Guangxi, China, and found evidence for sympatry of *P. sinensis* and the new putative species from Jiangxi, China. We also discovered evidence for hybridization in turtle farms and for the occurrence of alien lineages in the wild (Zhejiang, China), highlighting the risk of genetic pollution of native stock. In the face of the large-scale breeding of *Pelodiscus*, we claim that the long-term survival of distinct genetic lineages and species can only be assured when an upscale market segment for pure-bred softshell turtles is established, making the breeding of pure lineages lucrative for turtle farms. Our findings underline that the diversity of *Pelodiscus* is currently underestimated and threatened by anthropogenic admixture. We recommend mass screening of genetic and morphological variation of Chinese softshell turtles as a first step to understand and preserve their diversity.

Keywords China · Genetic pollution · Hybridization · Japan · Korea · Reptile · Russia · Vietnam

Shiping Gong and Melita Vamberger contributed equally to this work.

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Introduction

The so-called Chinese softshell turtles (*Pelodiscus* spp.) play an outstanding economic role as a major food resource in China and other East and Southeast Asian countries. According to a brochure printed for the 8th World Congress of Herpetology in China, annually more than 340,000 t of Chinese softshell turtles are produced and 900 million hatchlings raised in Chinese turtle farms (Tian et al. 2016) that were mainly established in the 1980s (Bu et al. 2014). However, consuming and breeding these turtles have a long tradition, dating back to the Spring and Autumn Period, more than 2400 years ago (Kang and Yan 2000). Supposedly, this must have led to the admixture

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and hybridization of different genetic lineages (Ernst et al. 2000; Fritz et al. 2010; Bu et al. 2014). For instance, Japanese populations are thought to consist of native and introduced softshell turtles (Sato et al. 1997; Suzuki and Hikida 2014).

Chinese softshell turtles are widely distributed in East Asia, from the Amur and Ussuri Rivers in the Far East of Russia through Korea, Japan, and eastern, central, and southern China to southern Vietnam. In addition, they have been naturalized in many countries (TTWG 2017). However, through the millennium-long farming and translocation of turtles, the native distribution ranges of the individual species are likely to have been obscured. Based mainly on mitochondrial DNA (mtDNA), four species of *Pelodiscus* are currently recognized (Fritz et al. 2010; Stuckas and Fritz 2011; TTWG 2017), even though most taxa are difficult to diagnose morphologically. Only the large-sized northern species *Pelodiscus maackii*, showing a distinctive mottled pattern, is relatively easy to distinguish from other species. Vietnamese *Pelodiscus*, whose taxonomic identity is unclear, are much smaller and quite colorful (Fritz et al. 2010), whereas *Pelodiscus* from China are morphologically highly variable, small to medium-sized and patterned or uniformly colored.

Pelodiscus sinensis is thought to have the largest distribution range of all four species (TTWG 2017). Most Chinese records are assigned to this species, while *P. axenaria* and *P. parviformis* are known only from relatively small regions. *Pelodiscus axenaria* was originally described from Hunan, China (Zhou et al. 1991), and later recorded also from Guangxi, where it occurs obviously in sympatry with *P. parviformis* (Yang et al. 2011), a species that has been originally described from Guangxi and Hunan (Tang 1997). Recently, Vietnamese populations were assigned to *P. parviformis* (TTWG 2017). Integrative taxonomic analyses focusing on the external morphology and genetics are needed to clarify taxonomy and distribution of all taxa. The situation is further complicated because some Chinese authorities (Zhou et al. 2013, 2016; Zhang et al. 2015a, b, 2017a, b, 2018) distinguish various "strains" or "varieties" that may refer to distinct species, pure-bred captive lineages, hybrids, or special breeds like color variants.

Previous studies on the taxonomy of Chinese softshell turtles focused either on morphological comparisons (Chkhikvadze 1987; Tang 1997; Zhou et al. 1991) or relied largely on variation of mtDNA (Fritz et al. 2010; Stuckas and Fritz 2011; Suzuki and Hikida 2014; Dong et al. 2016). Unfortunately, the morphological studies did not contribute to a better understanding of variation. Yang et al. (2011) combined morphological data with evidence from mtDNA sequences, but their study was inconclusive with respect to morphology. In addition, microsatellite loci were characterized in several publications but not used for taxonomic purposes (Que et al. 2007; Li et al. 2010, 2016; Bu et al. 2011, 2014; Zhu et al. 2012; Ma et al. 2014; He et al. 2018), even though this would have been an ideal tool for inferring gene flow (Vamberger et al. 2015; Kindler et al. 2017).

For the present study, we examined a large sample of wild-caught and farmed *Pelodiscus* turtles using three mtDNA fragments and five nuclear loci for assessing their genetic variability and taxonomy. We examined in particular how our sample matches with previously described taxa and whether there is genetic evidence for hybridization. For the latter purpose, we analyzed mitochondrial and nuclear DNA sequences separately. Mitochondrial DNA is inherited in the maternal line and, thus, not recombining. Therefore, it is not affected by hybridization, while the biparentally inherited and recombining nuclear DNA (nDNA) is expected to show signatures of hybridization. Pure turtles from the wild should show concordant patterns of mtDNA and nDNA differentiation, while conflicts are expected when hybridization has occurred. We expected genetic evidence for hybridization especially for farm turtles because it seems likely that different genetic lineages and species of *Pelodiscus* are crossed there. This should be reflected by mismatches between differentiation patterns of mitochondrial and nuclear DNA.

Materials and methods

Ninety-four wild-caught and 51 farm-bred *Pelodiscus* were genetically examined over the past 10 years (Table S1); 38 of these were already used in two previous studies (Fritz et al. 2010; Stuckas and Fritz 2011). The remaining 107 turtles were sequenced for the present investigation (Table S1). As far as possible, for each new sample the same three mtDNA fragments as in Fritz et al. (2010) were studied, i.e., the partial 12S rRNA gene (400 bp), the cytochrome *b* gene (cyt *b*, 1140 bp) plus adjacent DNA coding for tRNA-Thr (31 bp), and the partial NADH dehydrogenase subunit 4 gene (ND4, 680 bp) plus adjacent DNA coding for tRNAs (177 bp). Laboratory procedures for the fragments containing the 12S and cyt *b* genes followed Fritz et al. (2010); for the fragment with the partial ND4 gene and adjacent DNA, Praschag et al. (2011). In addition to these mtDNA data, five nuclear loci were sequenced that are known to be variable and informative in turtles (Fritz et al. 2012; Spinks et al. 2013; Praschag et al. 2017), namely the 26S protease regulatory subunit 4 (P26S4, 750 bp), the oocyte maturation factor Mos (C-mos, 566 bp), the recombination activation gene 2 (Rag2, 673 bp), the RNA fingerprint fragment 35 (R35, 1036 bp), and the anonymous locus TB01 (679 bp), which most likely codes for the transmembrane protein with metallophosphoesterase domain. Laboratory procedures for the nuclear loci have been

described in detail in Praschag et al. (2017). If the material was still available, the same nuclear data were also generated for the samples used in Fritz et al. (2010).

Mitochondrial DNA was analyzed with the Maximum Likelihood (ML) approach implemented in RAxML 7.2.8 (Stamatakis 2006) and with Bayesian inference using MrBayes 3.2.1 (Ronquist et al. 2012). For phylogenetic analyses, the three mtDNA fragments were concatenated and aligned with previously published data from our group (Fritz et al. 2010; Stuckas and Fritz 2011; including GenBank sequences utilized there) using BioEdit 7.1.3.0 (Hall 1999). Sequences were individually checked for quality and the presence of stop codons in MEGA 7.0.21 (Kumar et al. 2016). Sequences of the same taxa as in Fritz et al. (2010) and Stuckas and Fritz (2011) were added as outgroups (*Apalone spinifera*, *Palea steindachneri*, *Rafetus euphraticus*), yielding an alignment of 2428 bp length and including data of 148 individuals (Table S1). The optimal partitioning scheme and substitution model were determined using PartitionFinder (Lanfear et al. 2012) and the linked branch lengths option, resulting in partitioning by gene and the GTR + G model across all partitions. Clade support under ML was assessed by bootstrapping, with multiple independent runs using both fast and thorough bootstrap algorithms. For Bayesian inference, two parallel runs (each with four chains) and default parameters were used. The chains ran for 10 million generations with every 100th generation sampled. The calculation parameters were analyzed using a burn-in of 2.5 million generations to assure that both runs converged. Parameter convergence, sampling adequacy, and appropriate burn-in were determined using the software Tracer 1.6 (Rambaut et al. 2014). A 50% majority rule consensus tree was then generated from the posterior sample of trees, with the posterior probability of any individual clade corresponding to the percentage of all trees containing the respective clade.

Nuclear sequences were phased using the PHASE algorithm in DnaSP 5.10 (Librado and Rozas 2009). Alignments of each phased nuclear locus were examined using PopART (http://popart.otago.ac.nz) and the implemented parsimony network algorithm of TCS (Clement et al. 2000) for wild-caught and farmed turtles separately. Based on coded nuclear haplotypes, Principal Component Analyses (PCAs) were run using the R package adegenet 2.0.1 (Jombart 2008) to assess the distinctness of the mitochondrial genetic lineages on the genotypic level. Three different PCAs were calculated using the dudi.pca function: all samples ("ALL"), only samples from wild-caught turtles ("WILD"), and another PCA including only samples of farm-bred turtles ("FARM"). In additional PCAs, the data of the most divergent clusters were removed to examine whether the remaining data were better resolved.

Results

Mitochondrial DNA

Our phylogenetic analyses of mtDNA revealed 11 terminal clades within *Pelodiscus* (Fig. 1), some of which corresponded to those reported in previous studies (Fritz et al. 2010; Stuckas and Fritz 2011; Yang et al. 2011). A newly identified and maximally supported clade I was deeply divergent and sister to another maximally supported clade comprising the sequences of *Pelodiscus axenaria*. These two clades together constituted the sister group of the remaining *Pelodiscus* species. Among these, sequences identified as *P. parviformis* (Stuckas and Fritz 2011; Yang et al. 2011) constituted a maximally supported clade that was sister to a weakly to moderately supported crown group containing sequences of *P. maackii*, *P. sinensis*, and two individuals (clades G and H) grouping with neither of these species. The placement of clades G and H was badly resolved under both phylogenetic methods. All sequences of *P. maackii* corresponded to a maximally supported clade. It was sister to a more inclusive clade containing clades G and H plus all five clades within *P. sinensis* (clades B-F). One clade within *P. sinensis* was unknown before (clade E), and each of the five clades within *P. sinensis* was well supported. The sequence of the name-bearing lectotype for *P. sinensis* (Stuckas and Fritz 2011) clustered in clade F. The only two differences between ML and Bayesian topologies referred to weakly supported clades with short branch lengths. While both approaches placed clades G and H outside *P. sinensis*, these two clades were the successive sister taxa to *P. sinensis* under ML (bootstraps 17 and 40, respectively). In contrast, using Bayesian inference, clades G and H clustered together (weak support of 0.78), and this clade G + H was with high support (0.99) sister to *P. sinensis*. Within *P. sinensis*, ML suggested clade E as sister to a weakly supported clade B + (C + (F + D)), whereas the placement of clades B and E was swapped under Bayesian inference.

The geographic distribution of the mitochondrial lineages (clades) of wild-caught *Pelodiscus* and the provenance of farm-bred Chinese softshell turtles are shown in Fig. 2. The mitochondrial lineage of *P. axenaria* included, besides GenBank sequences from turtles from Guangxi and Hunan (Chen et al. 2005, 2006; Yang et al. 2011), data for four new wild-caught turtles from Longsheng (Guangxi), another wild-caught individual from Shixing (Guangdong), and one putatively farm-bred turtle bought on the Lianshan market (Guangdong). The newly discovered sister lineage of *P. axenaria* (clade I) corresponded to sequences from six wild-caught turtles from Gangkou, Fengxin (Jiangxi). *Pelodiscus parviformis* was represented by GenBank data (wild turtles from Guangxi and farm turtles) and new sequences of farmed turtles and from wild-caught individuals from Yongzhou (Hunan), Kaihua

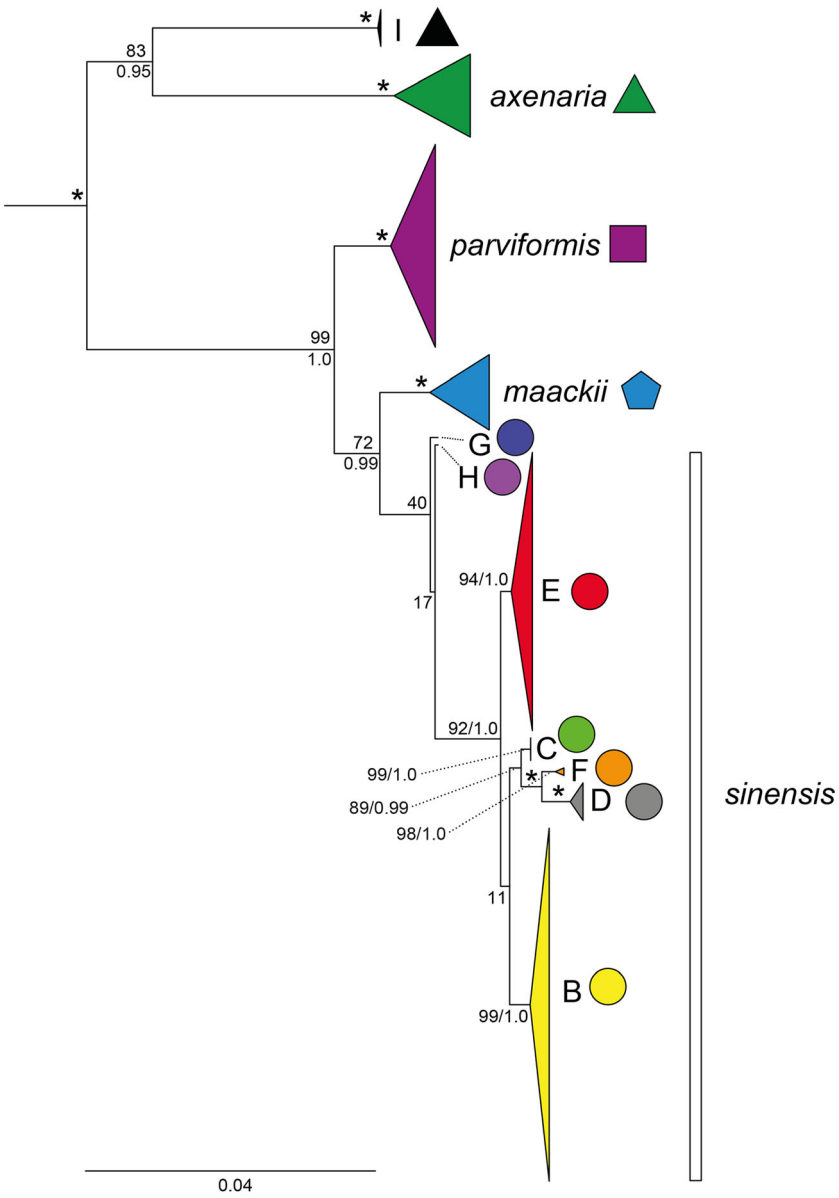


Fig. 1 Maximum Likelihood tree for mtDNA sequences of 145 Chinese softshell turtles (2428 bp). Outgroups (*Apalone spinifera*, *Palea steindachneri*, *Rafetus euphraticus*) removed for clarity. Numbers at nodes are bootstrap values (1000 replicates) and posterior probabilities from Bayesian inference. Asterisks indicate maximum support under both approaches. Lettering of clades B-C follows Fritz et al. (2010) and Stuckas and Fritz (2011); other clades (except for F, see text) were newly identified in the present study. Sequences of the lectotype of *Trionyx* (*Aspidonectes*) *sinensis* Wiegmann, 1834 = *Pelodiscus sinensis* (Stuckas and Fritz 2011) cluster in clade F. Clade symbols correspond to Fig. 2

(Zhejiang), and Longsheng (Guangxi). *Pelodiscus maackii* included previously published sequences from turtles from the Russian Far East (Fritz et al. 2010) and Korea (Jung et al. 2006) and a new wild-caught turtle from Kaihua (Zhejiang). Clades G and H, both placed outside *P. sinensis*, corresponded to one turtle each from Kaihua (Zhejiang). Within *P. sinensis*, clade B comprised many, in part previously published, sequences from farm-bred individuals and from wild-caught turtles from Ruyuan, Meizhou, and Zijin (all Guangdong) and from one turtle each from the Da River and the Ma River (northern Vietnam). Clade B was also found in wild-caught *Pelodiscus* from Okinawajima, Japan, which are not native according to Sato et al. (1997). Clade C contained only the previously published sequences from two putatively farm-bred market turtles and one wild-caught individual from Changlingzhen (Zhenba, Shaanxi; Fritz et al. 2010). Clade D included besides previously published sequences from turtles from central and southern Vietnam (Fritz et al. 2010) those from two individuals from the Da River in northern Vietnam. The newly discovered clade E was found in putatively farmed turtles from markets and in many wild-caught turtles from Dongyuan near Caicunzhen (Anhui), Lianzhou (Guangdong), Gangkou, Fenxin (Jiangxi), and Kaihua (Zhejiang). Some, but not all, of the wild-caught softshells from Gangkou, Fengxin represented the "black turtle" morphotype known in China. Clade F corresponded to sequences for only two turtles: one was a farm individual and the other the lectotype for *P. sinensis* (Stuckas and Fritz 2011) from the vicinity of Macau (Macao).

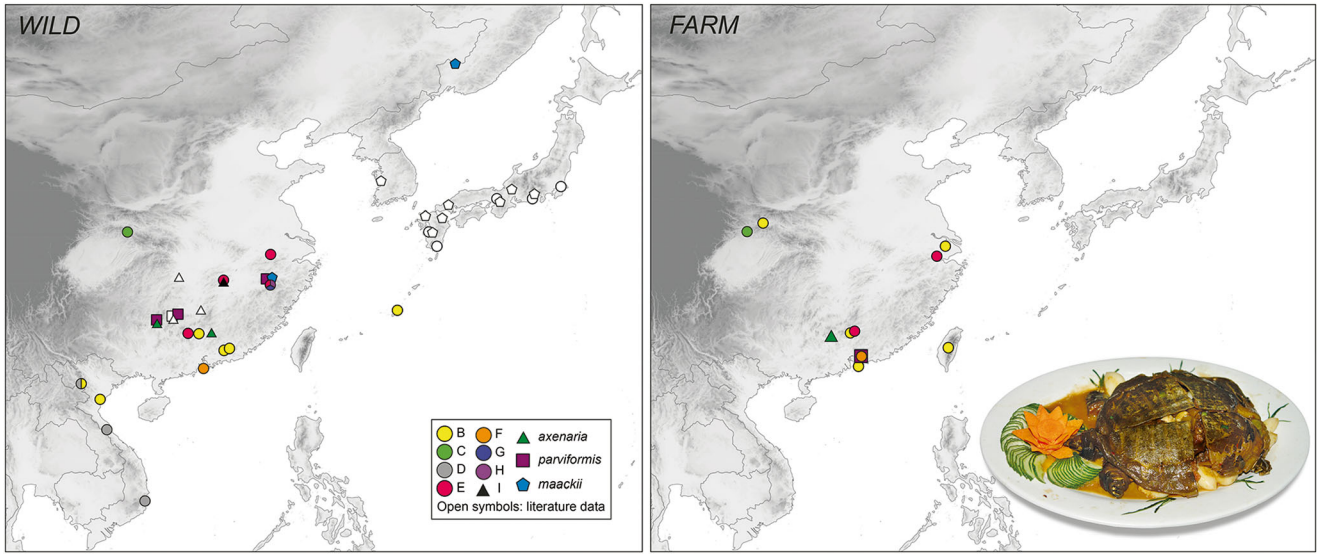


Fig. 2 Geographic distribution of mitochondrial lineages of wild-caught *Pelodiscus* according to the present study (WILD), including genetically verified records for *Pelodiscus axenaria* and *P. parviformis* from Chen et al. (2005, 2006) and Yang et al. (2011), for *P. maackii* from Jung et al. (2006). On the Japanese main islands Kyushu and Honshu, turtles harboring haplotypes resembling those of *P. maackii* are widely distributed and co-occur there with turtles yielding haplotypes of *P. sinensis*. The more abundant *maackii*-like haplotypes suggest that this lineage might be native (Suzuki and Hikida 2014). *Pelodiscus sinensis* in Okinawa Prefecture, Japan, are thought to represent post-World War II introductions from Taiwan (Sato et al. 1997). On the right (FARM), the location of the sources for farm-bred *Pelodiscus* is shown. Divided circles indicate the syntopic occurrence of the respective lineages. Inset: traditional Chinese turtle dish (photo: M. Auer)

Nuclear DNA

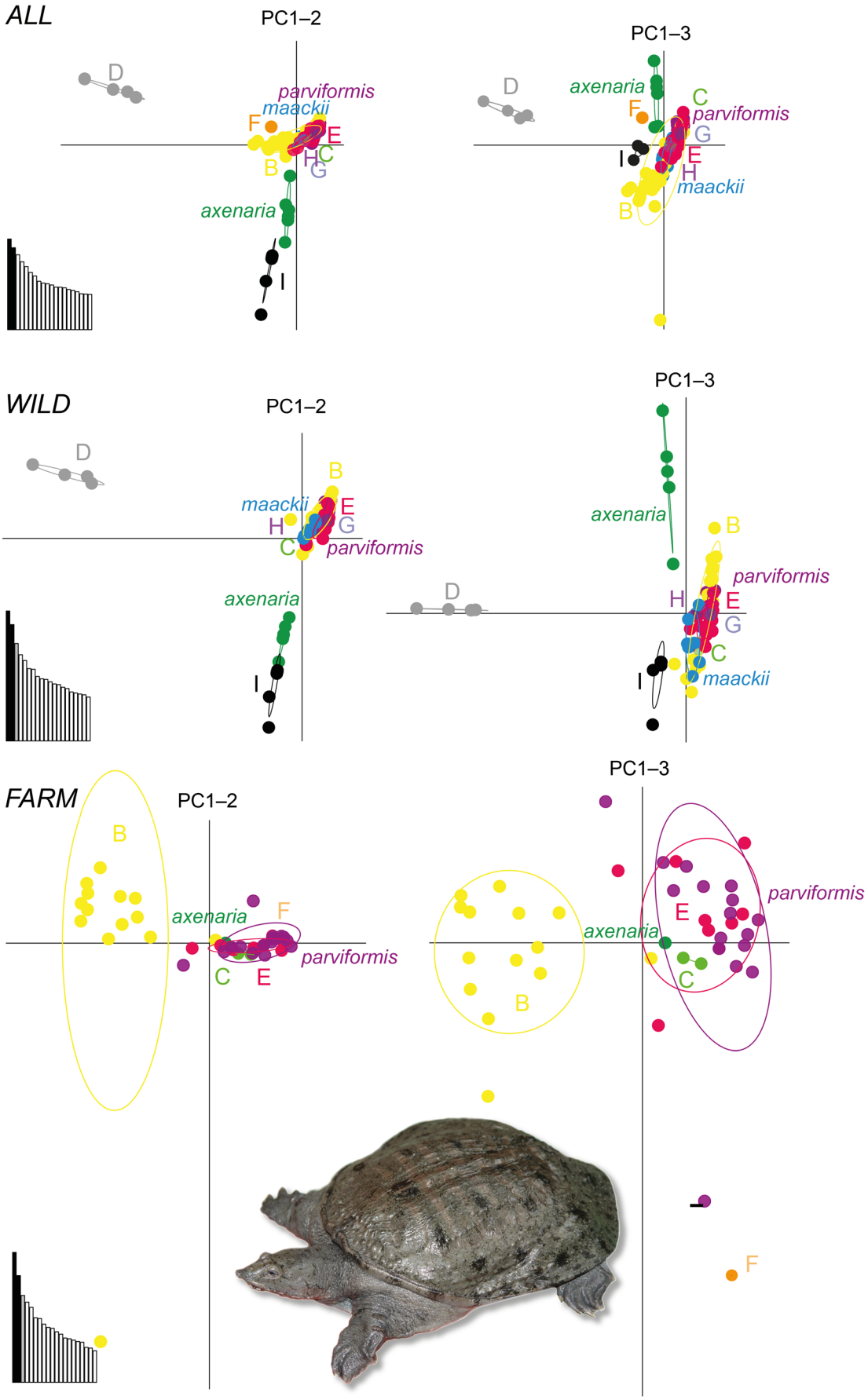
The networks of wild-caught and farmed *Pelodiscus* showed a high degree of haplotype sharing among the different genetic lineages and putative species (Fig. S1). Private haplotypes occurred in most lineages and species. However, the newly discovered lineage I had for three loci (R35, Rag2, P26S4) unique haplotypes and shared no haplotypes with other lineages or species. *Pelodiscus axenaria* and lineage D were also more distinct than others.

The PCA using information from all nuclear loci consisted for all samples of four clearly distinct clusters (Fig. 3: ALL). One cluster each corresponded to *Pelodiscus axenaria*, lineage D, and lineage I. The fourth cluster consisted of all remaining lineages and putative species, including lineages G and H that were placed in phylogenetic analyses of mtDNA outside *P. sinensis* (Fig. 1). Another PCA without the data of the most divergent clusters (*P. axenaria*, lineages D and I) showed no better resolution in the remaining data set (Fig. S2: ALL). For wild-caught *Pelodiscus* only (Fig. 3 and Fig. S2: WILD), similar results were obtained. For farm-bred *Pelodiscus*, lineage B corresponded to a cluster distinct from lineages C, E, F, and turtles harboring mitochondrial haplotypes of *P. axenaria* and *P. parviformis* (Fig. 3: FARM). A PCA using all farm turtles except lineage B resulted in one massively overlapping cluster for all samples except F (Fig. S2: FARM). In both of the latter PCAs, the only putatively farm-bred turtle with the mitochondrial lineage of *P. axenaria* clustered within samples of *P. parviformis* and *P. sinensis*.

Discussion

Our data clearly showed that the mitochondrial variation within *Pelodiscus* is still incompletely known. We discovered four new mitochondrial lineages (E, G, H, I). Two of these lineages (G, H) were represented by only one individual each. In phylogenetic analyses, G and H clustered outside *Pelodiscus sinensis*, but their placement was weakly resolved (Fig. 1). In PCAs using five nuclear loci, they clustered together with *P. maackii*, *P. parviformis*, and most mitochondrial lineages of *P. sinensis* (Fig. 3). Further sampling with more nuclear loci is necessary to disentangle their relationships. We explicitly do not assign the two individuals with lineage G and H haplotypes to any species. Another new mitochondrial lineage (lineage I) was highly distinct and sister to *P. axenaria* (Fig. 1). This lineage and *P. axenaria* were also clearly distinct when PCAs of the five nuclear loci were calculated (Fig. 3), supporting the species status of *P. axenaria* and suggesting that lineage I represents another unknown species of *Pelodiscus*. In contrast, in the PCAs *P. maackii* and *P. parviformis* were not found to be differentiated from most mitochondrial lineages of *P. sinensis*. However, since we used only five nuclear loci, this does not necessarily contradict their status as distinct species and further research is warranted to elucidate this situation.

According to our data, several mitochondrial lineages of *Pelodiscus* co-occur in the wild (Fig. 2). Putative *P. axenaria* were caught together with putative *P. parviformis* near Longsheng (Guangxi, China). This


ALL
PC1–2
PC1–3
D
parviformis
maackii
axenaria
F
E
C
H
G
B
I
WILD
FARM

◀ **Fig. 3** Principal Component Analyses (PCAs) using coded phased haplotypes of all five nuclear loci of wild and farm-bred Chinese softshell turtles (ALL), wild turtles only (WILD), and farm-bred turtles only (FARM). Color-coding corresponds to mitochondrial lineages of Fig. 1. Ovals represent 95% confidence intervals. For ALL, the first, second, and third principal components (PCs) explain 62, 57, and 49% of the observed variance; for WILD, 65, 58, and 49%; and for FARM, 64, 53, and 43%, respectively. For PCAs without the most divergent clusters, see Fig. S2. Inset: farm-bred *Pelodiscus sinensis* (lineage B, sample 5084; photo: M. Auer)

confirms the earlier reported sympatric occurrence of these two species in Guangxi (Yang et al. 2011). While the PCAs of our nuclear markers did not support the distinctness of wild-caught *P. parviformis* from wild turtles yielding mtDNA haplotypes identified with *P. sinensis*, the syntopically occurring *P. axenaria* from Longsheng were distinct in nuclear markers from all other *Pelodiscus* (Fig. 3), including *P. parviformis*. This further supports the recognition of *P. axenaria* as a distinct species. Until now, *P. axenaria* was only known from Guangxi and Hunan, China (TTWG 2017). We reported now for the first time a wild-caught *P. axenaria* from the Chinese province of Guangdong (Shixing). It remains unclear whether this record represents a translocated or a native individual. Shixing lies between sites for lineage B of *P. sinensis*, suggestive of a sympatric occurrence with *P. axenaria*.

Moreover, softshells with the new and highly distinct mitochondrial lineage I were caught together with *P. sinensis* (lineage E), some of the latter representing a black morphotype, at Gangkou, Fengxin in Jiangxi province, China. In the PCAs of nuclear markers, lineage E clustered with other mitochondrial lineages of *P. sinensis*, *P. maackii*, and *P. parviformis*. Yet, it was distinct from the sympatrically occurring lineage I turtles, corroborating that softshells with lineage I represent an unknown species.

A remarkable finding was that *Pelodiscus* with mtDNA lineage D were highly distinct from all others based on PCAs of nuclear loci (Fig. 3), even though their mitochondrial lineage is only weakly differentiated from other lineages identified with *P. sinensis*, *P. maackii*, and *P. parviformis* (Fig. 1). In contrast to lineage D, the remaining lineages were only feebly differentiated or undifferentiated in the examined nuclear markers (with the possible exception of lineage B; Fig. 3; Fig. S2). Turtles with lineage D are only known from Vietnam and were caught together with putative *P. sinensis* of lineage B in the Da River (Fig. 4). The same lineage of putative *P. sinensis* was also caught in the Ma River (Table S1), suggesting that the occurrence of lineage B in northern Vietnam could be native and not due to released or escaped farm turtles. If *P. sinensis* occurs there naturally, the syntopic record of turtles with lineage D, both distinct in nuclear markers (Fig. 3) and morphology (Fig. 4), would indicate species status for both. In this case, it can only be speculated why the mitochondrial lineage D of the Vietnamese turtles is so weakly differentiated. One possibility is mitochondrial capture, also known in other turtle species (Fritz et al. 2008; Ihlow et al. 2016; Praschag et al. 2017; Vamberger et al. 2017). In any case, we doubt that the *Pelodiscus* populations from Vietnam harboring lineage D will be identified with *P. parviformis*, as recently suggested (TTWG 2017). Based on coloration similarities and the small size of Vietnamese turtles, Fritz et al. (2010) indicated that these southern populations resemble the description of *P. parviformis*. As a consequence, Vietnam has been tentatively included as a disjunct area into in the distribution range of *P. parviformis* (TTWG 2017). However, mtDNA sequences from softshells from Guangxi identified with *P. parviformis* (Yang et al. 2011; this study) are distinct from those of Vietnamese turtles (Fritz et al. 2010; this study; Fig. 1), rendering their conspecificity very unlikely.

We recorded the greatest diversity of sympatrically occurring mitochondrial lineages in China's Zhejiang province (Kaihua). There, we found no less than five distinct lineages corresponding to three putative *Pelodiscus* species (*P. maackii*, *P. parviformis*, *P. sinensis*; Fig. 2; Table S1). A haplotype from Kaihua matching with *P. maackii* was the only record for this species in China and more than 1200 km away from its native range in the Russian Far East and Korea. *Pelodiscus maackii* reaches a much larger size than the other *Pelodiscus* species (according to Brandt 1857 up to 35 cm shell length; according to Przewalskii cited in Kuzmin 2002 even 45 cm, compared to maximum sizes of 15 cm in *P. parviformis* and 20 cm in *P. axenaria* and *P. sinensis*; Zhou et al. 1991; Tang 1997; Fritz et al. 2010; Yang et al. 2011; S. Gong unpubl. data). The large adult size makes breeding *P. maackii* attractive, also for crossbreeding to boost offspring size, so we assume that this species and its hybrids are frequently kept in Chinese farms. Thus, it seems likely that the turtle from Kaihua or its ancestors escaped from a farm. Among the five lineages recorded in Kaihua were also the only records of the newly identified lineages G and H, each represented by only one individual. It is unclear whether one or both of these lineages occur naturally there. As shown by the record of the mitochondrial lineage of *P. maackii*, the unusual diversity in Kaihua exemplifies genetic contamination through escaped or intentionally introduced turtles. We are convinced that this is a frequent issue obscuring more and more natural distribution patterns. Moreover, alien *Pelodiscus* most likely hybridize with native stock and endanger, together with crossbreeding in turtle farms, the genetic integrity of pure taxa and lineages. Such genetic pollution and erosion of diversity is common in animals and plants exploited as food resources, for fishing, hunting, logging (Laikre et al. 2010), or in animals used for pets, and should be closely monitored. Hybridization and genetic pollution are known to be an issue also for some other turtle species (e.g., *Emys orbicularis*, Vamberger et al. 2015; Raemy et al. 2017; *Mauremys*

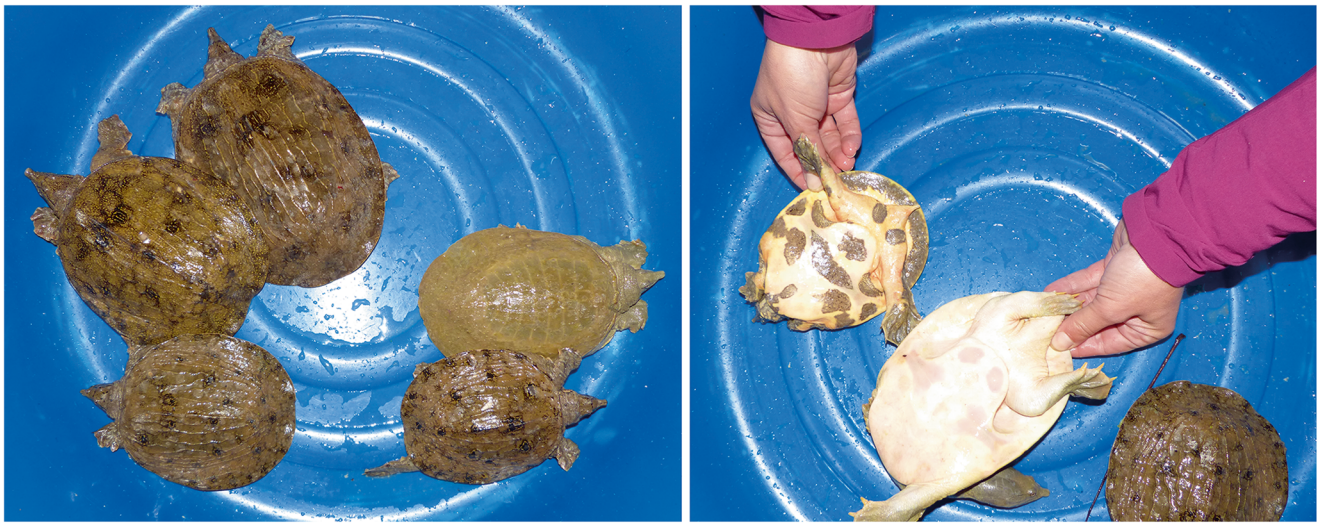


Fig. 4 Syntopically caught *Pelodiscus* from the Da River, northern Vietnam. The plain-colored individual yielded mitochondrial lineage B of *Pelodiscus sinensis*. The spotted turtles correspond to the morphotype associated with lineage D, and two individuals were genetically examined and harbored this lineage (photos: P. Praschag)

reevesii and *M. sinensis*, Fong and Chen 2010; *M. japonica* and *M. reevesii*, Suzuki et al. 2014).

Our sample of farm-bred *Pelodiscus* does not include all genetic lineages we recorded from the wild. However, we found in one market turtle from Guangdong, China, a mitochondrial haplotype of *P. axenaria*. Unlike the wild-caught *P. axenaria*, which were distinct in PCAs using nuclear loci, this Guangdong turtle clustered within farm-bred *P. parviformis* and *P. sinensis* (Fig. 3), suggestive of interspecific hybridization in breeding facilities. Farm hybrids are also known for other turtle species (Parham et al. 2001; Stuart and Parham 2007), even though the concerned taxa are bred only for a few decades commercially (Tian et al. 2016). Thus, a much larger extent of hybridization is expected for *Pelodiscus* with its millennium-old breeding tradition. In the face of the massive large-scale breeding of *Pelodiscus*, we believe that only clever marketing strategies can ensure the long-term survival of distinct genetic lineages and species. In an increasingly wealthy China, there is already now a market for "bioturtles," i.e., *Pelodiscus* raised under conditions complying with organic food production. Such bioturtles are sold along with regularly raised softshells, but for more money, in supermarkets. If another upscale market segment for pure-bred "varieties" of Chinese softshell turtles could be established, a window will be opened for the long-term conservation of genetic lineages and species that will otherwise disappear through mass-breeding of a uniform standard turtle.

Further research is warranted to elucidate the genetic variation within *P. sinensis* and of the enigmatic turtles with haplotypes of the divergent mitochondrial lineages G and H, clustering outside *P. sinensis* in phylogenetic analyses. With respect to *P. sinensis*, the sympatric occurrence of lineages B and D in northern Vietnam deserve special attention. In addition, turtles harboring mitochondrial lineage B appear quite distinct in PCAs using our five nuclear loci. However, this seems to be largely due to farm-bred individuals (Fig. 3), and we can exclude neither that the differentiation of these farm turtles results from hybridization nor that this represents merely a sampling bias. More research will also lead to a better understanding of taxonomy, natural gene flow across taxa, human-mediated hybridization, and distribution patterns of distinct species and genetic lineages. For examining gene flow and hybridization, a toolbox of microsatellite loci is already available (Que et al. 2007; Li et al. 2010, 2016; Bu et al. 2011, 2014; Zhu et al. 2012; Ma et al. 2014; He et al. 2018), and new genomic approaches open unprecedented possibilities for future research. A challenge will be to obtain dense range-wide sampling of wild *Pelodiscus*, while hybridization and differentiation of farm stock should be relatively easy to study.

In conclusion, our results show that the diversity of *Pelodiscus* is currently underestimated and threatened by genetic pollution. To understand and preserve the diversity of Chinese softshell turtles, we recommend mass screening of their genetic and morphological variation to lay the foundations for future conservation actions.

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Author contributions SG and UF conceived and designed research. SG, MA, and PP collected samples. MV analyzed data. UF, MV, and SG wrote the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study is not based on any experiments with humans or animals. Samples for genetic investigation were taken minimally invasive according to all applicable international and national guidelines, regulations, and best ethical and experimental practice of the Senckenberg Nature Research Society.

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