BMC
Research Notes

RESEARCH ARTICLE   **Open Access**

Mitochondrial and morphological variation of *Tilapia zillii* in Israel

Amir Szitenberg, Menachem Goren and Dorothée Huchon[*]

Abstract

Background: *Tilapia zillii* is widespread in the East Levant inland aquatic systems as well as in artificial water reservoirs. In this study we explore the genetic and morphological variation of this widespread species, using mitochondrial control region sequences and meristic characters. We examine the hypothesis that *T. zillii*'s population structure corresponds to the four Israeli aquatic systems.

Results: Out of seven natural water bodies, only two were found to possess genetically divergent populations of *T. zillii*. In addition to its presence in fish farms, the species was found in two artificial recreational ponds which were supposed to have been stocked only with other fish species. In these two artificial habitats, the haplotype frequencies diverged significantly from those of natural populations. Finally, fish from the Dead Sea springs of Ne'ot HaKikar appear to differ both genetically and morphologically from fish of the same aquatic system but not from fish of other water systems.

Conclusions: Our results show that the population structure of *T. zillii* does not match the geography of the Israeli water-basins, with the exception of the Dead Sea and Kishon River, when considering natural populations only. The absence of a significant divergence between basins is discussed. Our results and observations suggest that the Ne'ot HaKikar Dead Sea population and those of artificial ponds could have originated from the "hitchhiking" of *T. zillii*, at the expense of some other cultivated tilapiine species.

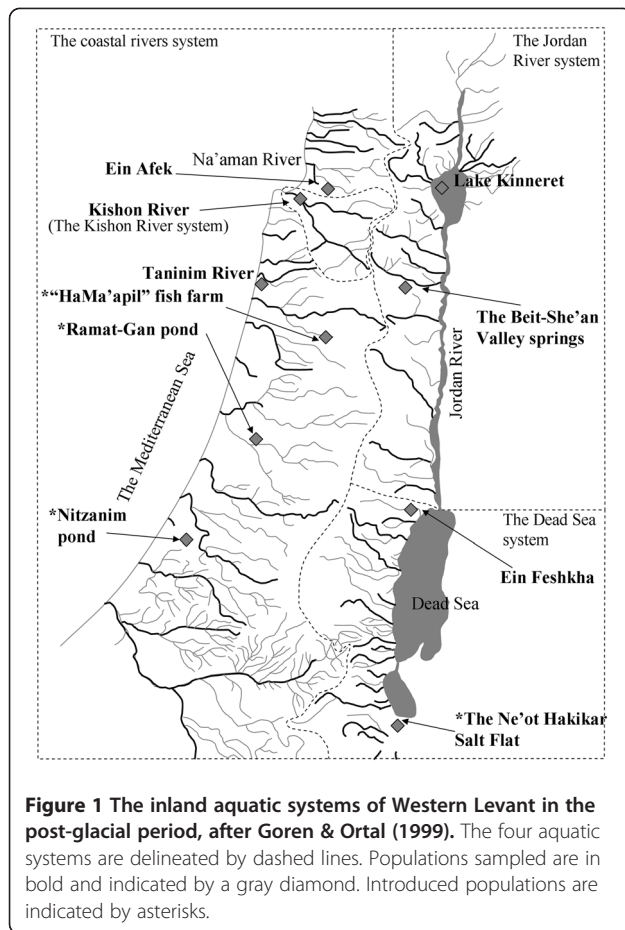**Keywords:** *Tilapia zillii*, Population genetics, Mitochondrial control region, Meristic counts

Background

Tilapia zillii (Gervais, 1848) is an African and Middle-Eastern native tilapiine fish [1]. The Israeli populations represent the periphery of Tilapiinae natural geographic distribution. Such peripheral populations may have a high evolutionary significance, as range peripheries are thought to constitute some of the most important areas for speciation [2]. Present-day Israel has three geological aquatic systems (Figure 1): the coastal, the Jordan River, and the Dead Sea systems. Based on faunal composition, a fourth "system", the Kishon River, can be considered separately [3]. *T. zillii* is present in all four systems. Being euryhaline, *T. zilli* is able to extend its geographic distribution into habitats of a wide salinity range. Specimens have been observed in estuaries and even in shallow marine habitats along the Mediterranean coast [4]. *T. zillii*'s euryhalinity is thus considered to be a key reason for

the wide geographic distribution of the species [5,6], in particular in coastal rivers. *T. zillii* is a monogamous biparental guarder and substrate brooder with both parents committed to a single nest through a breeding cycle [7].

Interestingly, *T. zillii* has been found in artificial recreational water reservoirs created in the 1960-70s (e.g., the Ramat-Gan Urban Park and the Nitzanim Nature Reserve, Figure 1). These artificial reservoirs are isolated water bodies that are not connected to any natural habitat. They were stocked with other fish species, in particular tilapiine fishes, from aquacultural supplies. Since *T. zillii* is not listed as a cultivated species in Israel [8], it is not expected to be present in these ponds, which have been populated solely with aquaculture stocks and are isolated from natural water systems. Similarly, in the Dead Sea area, *T. zillii* has been known to be present in the northern springs of the system (e.g., Ein Feshkha, Figure 1) [7]. In the last 20 years the fish has also been detected in the southern section of the Dead Sea system,

* Correspondence: huchond@post.tau.ac.il
Department of Zoology, Tel-Aviv University, Tel Aviv 69978, Israel

Figure 1 The inland aquatic systems of Western Levant in the post-glacial period, after Goren & Ortal (1999). The four aquatic systems are delineated by dashed lines. Populations sampled are in bold and indicated by a gray diamond. Introduced populations are indicated by asterisks.

in the Ne'ot HaKikar area (Figure 1). The origin of these recent populations is unknown. Our study seeks to understand *T. zillii*'s mitochondrial and morphological variations in Israel and its population structure, using the control region sequence and meristic characters as markers.

Results

T. zillii was sampled from two coastal system habitats (Ein Afek Nature Reserve and the Taninim River), the Kishon River, two Jordan River habitats (Lake Kinneret and Beit She'an Valley springs) and two Dead Sea system habitats (Ein-Feshkha Nature Reserve and the Ne'ot HaKikar Salt Flat). In addition, two artificial recreational ponds located in the coastal aquatic system were sampled (the Ramat-Gan Park pond and the Nitzanim Nature Reserve pond). All sample sites are indicated in Figure 1.

Sequence analysis
Eleven haplotypes were recognized among the 117 *T. zillii* D-loop sequences obtained (Table 1). The most widespread haplotype (one) was found in all samples except for the

Kishon River (Kishon system), Ein Feshkha (Dead Sea sytem), and Nitzanim (coastal introduced) samples. The two fish farm specimens also possessed this haplotype. The Ein Feshkha and Ne'ot HaKikar samples (both from the Dead Sea system) were monomorphic, but for different haplotypes (Table 1; Figure 2). The Kinneret sample (Jordan River system) showed greater haplotype diversity than any other ($h = 0.69$, sd = 0.12). As a consequence of the high haplotype diversity, the effective size estimation of this population is also higher than all the others ($\theta_s = 2.11$, sd = 1.05), with the Kinneret being the largest most continuous body of water examined. Among the artificial ponds' samples, that from Ramat Gan was the most diversified ($h = 0.56$, sd = 0.09). With the exception of the monomorphic Dead Sea samples, the Kishon River sample provided the smallest θ_s estimation ($\theta_s = 0.35$, sd = 0.35) (Table 1).

When including both natural and artificial habitat samples, AMOVA revealed that within-population variation explained about half (48.8 %) of the total genetic variation. The other approximate half of the variation (48.3 %) was explained by variation among populations within the various aquatic systems ($\Phi_{sc} = 0.50$, $P < 10^{-5}$). Variation among aquatic systems was thus small (2.9 %) and non significant ($\Phi_{ct} = 0.03$, $P = 0.35$). Pairwise Φ_{st} indices as well as Nei's corrected average numbers of pairwise difference (Table 2) indicated that samples from artificial ponds (Nitzanim and Ramat-Gan) significantly diverged from all other samples and from one another, including those from the coastal system (i.e., the natural system within which the artificial ponds were located). The sample from Ne'ot HaKikar (Dead Sea system) appeared to differ from the second Dead Sea sample (Ein Feshkha) but not from some samples from the Jordan River and coastal water systems. Taken together with its recent discovery, the population of Ne'ot HaKikar is suspected of being a recent introduction. These findings are reflected in the haplotype network (Figure 2).

When the three samples that are presumed to have been artificially introduced (Ne'ot HaKikar, Nitzanim and Ramat-Gan) were excluded from the AMOVA, a significant difference among some of the systems emerged ($\Phi_{ct} = 0.58$, $P = 0.013$). Analysis of Nei's corrected average numbers of pairwise differences and pairwise Φ_{st} indices revealed that the Dead Sea sample of Ein Feshkha differed from the others ($2.08 < D < 2.61$, $P < 10^{-5}$). This was also the case for the Kishon River ($1.46 < D < 2.08$, $P < 10^{-5}$). However, the divergence between the Jordan River and coastal systems was not significant ($D = 0.02$, $P = 0.25$).

Meristic counts
In the nested MANOVA of 22 meristic characters (Table 3) the factors "population within aquatic systems"

Table 1 Molecular diversity of *T. zillii* populations

	Aquatic system	Coastal					Kishon	Jordan River		Dead Sea	
	Population	Taninim	Ein Afek	Ramat Gan*	Nitzanim*	HaMa'apil*	Kishon	Kinneret	Beit She'an	Ein Feshkha	Ne'ot HaKikar*
Hap.	Polymorphic sites										
	111123357777										
	888882581455										
	234636718807										
1	GTGATAATACA-	11	8	1	0	2	0	9	7	0	15
2	..A..G.C...C	1	1	0	2	0	7	0	0	0	0
3	..A..G...-.-	1	0	9	0	0	3	2	5	0	0
4	......G....-	0	1	5	8	0	0	0	0	0	0
5	A..G.......-	0	0	0	0	0	0	1	0	0	0
6	A..........-	0	0	0	0	0	0	1	0	0	0
7	.C.........-	0	0	0	0	0	0	1	0	0	0
8	..........G-	0	0	0	0	0	0	1	0	0	0
9	..A..G..G-.-	0	0	0	0	0	0	1	0	0	0
10	.......C.-.-	0	0	0	0	0	0	0	1	0	0
11	..A.C..C.-.-	0	0	0	0	0	0	0	0	13	0
	n	13	10	15	10	2	10	16	13	13	15
	h	0.29±0.16	0.38±0.18	0.56±0.09	0.35±0.16	0	0.47±0.13	0.69±0.12	0.6±0.09	0	0
	Π	0.001±0.001	0.001±0.001	0.002±0.001	0.002±0.001	0	0.002±0.00	0.002±0.001	0.002±0.001	0	0
	Θ_s	0.97±0.001	1.41±0.86	0.92±0.6	1.41±0.86	0	0.35±0.35	2.11±1.05	0.97±0.63	0	0
	Θ_π	1.03±0.82	1±0.83	2.02±1.35	1.77±1.26	0	1.4±1.06	1.71±1.18	1.72±1.2	0	0

From left to right: haplotype number (Hap.), the polymorphic sites in the Control region sequence (polymorphic sites), the number of occurrences of each haplotype in each sample. Diversity parameters : n = sample size, h = haplotype diversity, π = nucleotide diversity, Θ_s and Θ_π -estimates of population effective size multiplied by the mutation rate. *Introduced population.

and "aquatic system" significantly partitioned the variance of the meristic counts ($F_{105,\ 538} = 4.19$, $P < 10^{-5}$). MANOVA results were not affected by the inclusion or removal of samples from the introduced populations. A scatterplot of the first and second discriminant functions (accounting for 36% and 25.3% of the variation, respectively) clearly separated the Ein Feshkha sample from all others. In this plot (Figure 3), individuals of the coastal sample from Ein Afek and the (probably introduced) sample from Ne'ot HaKikar partly overlap. The highest correct classification rates, according to meristic counts, were of the Ein Feshkha sample (93.8%) and the
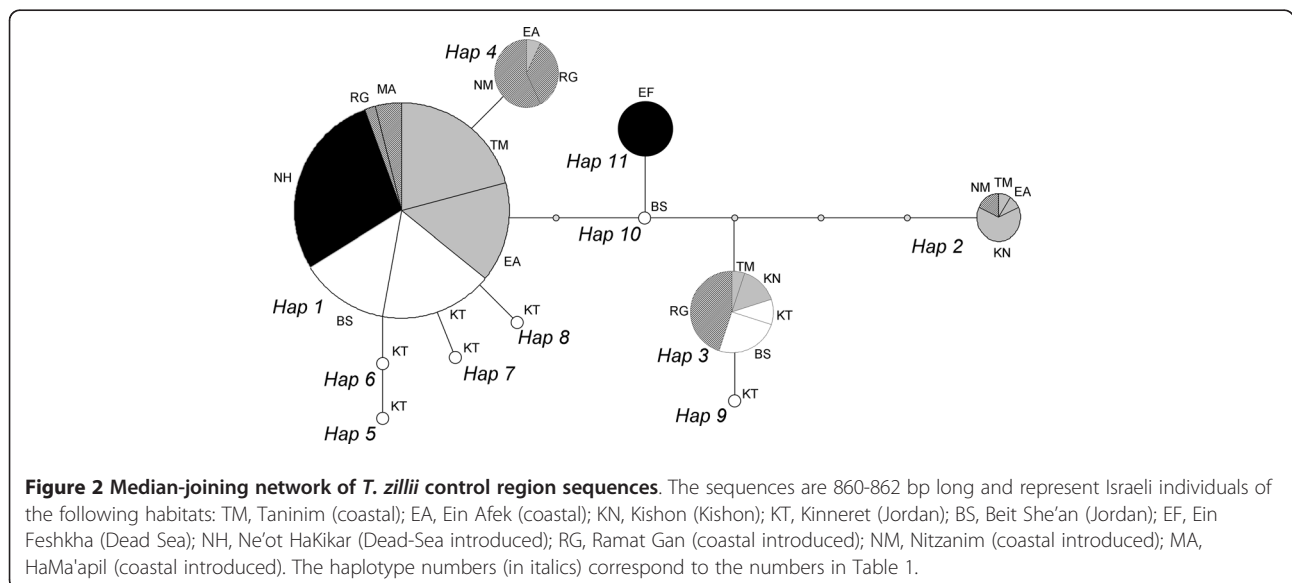

Figure 2 Median-joining network of *T. zillii* control region sequences. The sequences are 860-862 bp long and represent Israeli individuals of the following habitats: TM, Taninim (coastal); EA, Ein Afek (coastal); KN, Kishon (Kishon); KT, Kinneret (Jordan); BS, Beit She'an (Jordan); EF, Ein Feshkha (Dead Sea); NH, Ne'ot HaKikar (Dead-Sea introduced); RG, Ramat Gan (coastal introduced); NM, Nitzanim (coastal introduced); MA, HaMa'apil (coastal introduced). The haplotype numbers (in italics) correspond to the numbers in Table 1.

Table 2 Pairwise population divergence

	1	2	3	4	5	6	7	8	9
1: Taninim (coastal)		$<10^{-5}$ ns	0.44^{***}	0.56^{***}	1.39^{***}	$<10^{-5}$ ns	0.03^{ns}	2.58^{***}	0.03^{***}
2: Ein Afek (coastal)	$<10^{-5}$ ns		0.47^{**}	0.41^{***}	1.53^{***}	$<10^{-5}$ ns	0.09^{ns}	2.62^{***}	$<10^{-5}$ ns
3: Ramat Gan (coastal*)	0.30^{***}	0.33^{**}		0.46^{**}	0.62^{***}	0.39^{***}	0.11^{**}	2.60^{***}	0.78^{***}
4: Nitzanim (coastal*)	0.29^{***}	0.22^{***}	0.30^{**}		1.74^{**}	0.60^{***}	0.61^{**}	2.91^{***}	0.70^{***}
5: Kishon (Kishon)	0.55^{***}	0.56^{***}	0.35^{***}	0.52^{**}		1.4^{***}	0.78^{***}	1.94^{***}	2.03^{***}
6: Kinneret (Jordan)	$<10^{-5}$ ns	$<10^{-5}$ ns	0.22^{***}	0.27^{***}	0.50^{***}		0.03^{ns}	2.70^{***}	0.06^{ns}
7: Beit She an (Jordan)	0.10^{ns}	0.16^{ns}	0.05^{**}	0.32^{**}	0.40^{***}	0.04^{ns}		2.35^{***}	0.26^{**}
8: Ein Feshkha (Dead Sea)	0.87^{***}	0.89^{***}	0.05^{**}	0.84^{***}	0.77^{***}	0.78^{***}	0.75^{***}		3.02^{***}
9:Ne'ot HaKikar (Dead Sea*)	0.06^{ns}	0.04^{ns}	0.53^{***}	0.52^{***}	0.77^{***}	0.08^{ns}	0.37^{**}	1^{***}	

Above diagonal: Nei's corrected average numbers of pairwise differences. Below diagonal: pairwise Φ_{st} indices. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ^{ns} non-significant. Introduced populations are indicated by an asterisk.

Kishon sample (90%), while the percentage of correct classification of the other samples ranged from 56 to 83. Two characters were found to influence the first and second discriminant functions, respectively: the position of the longest pectoral ray; and the number of scales between the lower lateral line and the anal fin. The mean values and standard deviation of these characters in each sample are presented in Table 4.

Table 3 Meristic characters considered

Code	Meristic count
LL1	Number of scales along the upper lateral line
LL2	Number of scales along the lower lateral line
TR1	Number of scales between the dorsal fin and the upper lateral line
TR2	Number of scales between the upper and lower lateral line
TR3	Number of scales between the lower lateral line and the anal fin
P	Number of rays in the pectoral fin
Pbr	Number of branched rays in the pectoral fin
V	Number of rays in the ventral fin
Vbr	Number of branched rays in the ventral fin
D	Number of rays in the dorsal fin
Dbr	Number of branched rays in the dorsal fin
DS	Number of spikes in the dorsal fin
A	Number of rays in the anal fin
Abr	Number of branched rays in the anal fin
AS	Number of spikes in the anal fin
C	Number of rays in the caudal fin
Cbr	Number of branched rays in the caudal fin
LPR	The position of the longest pectoral ray
LVR	The position of the longest ventral ray
LDR	The position of the longest dorsal ray
LAR	The position of the longest anal ray
GR	The number of gill rakers

Discussion

Based on our results, the geological structure of the examined water systems do not account completely for the population structure of *T. zillii* in Israel. Two natural water bodies, Ein Feshkha (Dead Sea system) and the Kishon River, possess fish populations that are inferred to be isolated from the other water bodies. Conversely, fishes from the two major water systems, the coastal rivers system and that of the Jordan River, could not be differentiated based either on molecular or morphological data.

The distinction of the Kishon River from the rest of the coastal rivers based on its ichthyofauna composition [3] is supported by the molecular and morphological data presented here. However, this difference may also be the result of a recent severe bottleneck as this river has been heavily polluted for several decades [9].

The isolation of the Ein Feshkha Dead Sea system natural population based on molecular and morphological data corresponds to the geological structure of the inland water systems of Israel, where the hypersaline water of the Dead Sea poses a biological barrier for most aquatic organisms between the Jordan River and the Dead Sea system [3], a barrier that has existed for 60,000 years [10]. Isolation by distance and drift are two additional effects that may explain the genetic and morphological divergence of this population, since it is geographically distant from all other sampling sites considered, and one of the smallest water bodies sampled.

The lack of isolation between the coastal rivers' water system and the Jordan River water system could be an artifact of the small sample-size considered in our analysis, which does not allow us to identify significant differences. In particular, it is highly likely that the populations of the Costal system and the Jordan River basin are larger and less affected by drift than the populations present in the small Dead Sea water bodies or the isolated artificial ponds. Another explanation for the lack of isolation between the coastal system and the Jordan
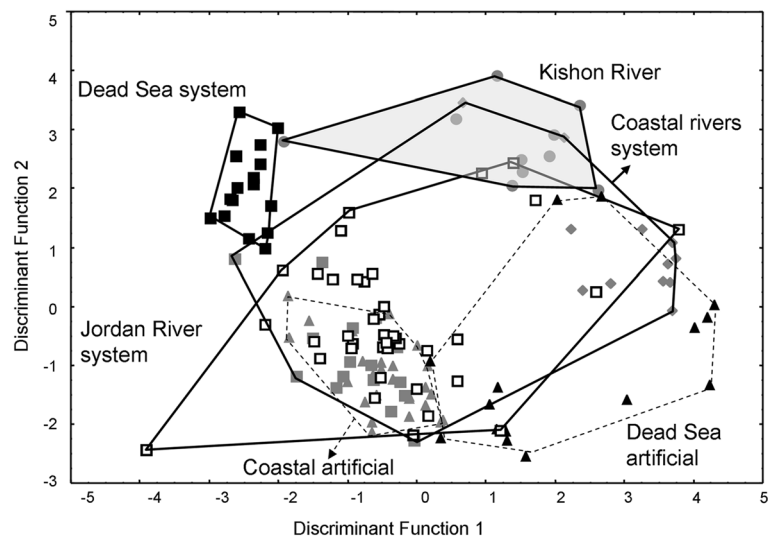
Figure 3 Discriminant analysis of *T. zillii* meristic counts. Individuals are from the following groups: gray square, coastal; gray diamond, Ein Afek (coastal); white square, Jordan; black square, Ein Feshkha (Dead-Sea); black triangle, Ne'ot HaKikar (Dead-Sea introduced); gray triangle, coastal introduced; gray circle, Kishon.

River system could be the translocation of *T. zillii* due to ancient aquaculture and trade [11-13].

Concerning the origin of fish present in artificial ponds, the most likely hypothesis is that these fish were introduced when the ponds were populated with fish farm stocks. Although *T. zillii* is not cultivated in Israel [8], it is found in fish farms [14]. Since there are no records of import of *T. zillii*, the individuals present in fish farms are thought to have colonized the fish farm pools during flooding. This is supported by the fact that the haplotypes present in the sampled artificial ponds do not differ from the haplotypes present in the natural water bodies, which suggests a local origin. In the case of the Ne'ot HaKikar (Dead Sea) population, the fact that the first *T. zillii* were spotted in that habitat following the establishment of a fish farm specializing in tilapiine stocks (M. G. personal observation), suggests that the fish might have escaped from the fish farm. This possibility is supported by the fact that another species, *Sarotherodon galilaeus*, is present in Ne'ot

HaKikar as a result of its escape from the same fish farm (M. G. personal observation, [15]). The hypothesis of an influence of the fish farm is also supported by the fact that *T. zillii* from Ne'ot HaKikar are more similar both genetically and morphologically to fish from the Ein Afek coastal river population than to those of the Ein Feshkha Dead Sea population.

Conclusions

The mitochondrial and morphological variability of the *T. zillii* population is only partially explained by the geographic distribution of the species in Israel, using the control region sequence as a molecular marker and meristic characters as morphological markers. The possibility that *T. zillii* infiltrates fish farms and might be transported unintentionally together with target species into new environments should be examined in more detail, since such translocation could have an impact on the fish diversity. Future studies based on additional

Table 4 Sample sizes, mean standard body length and the mean of key meristic counts

Aquatic system	Coastal					Kishon	Jordan River		Dead Sea	
Sample	Taninim	Ein Afek	Ramat Gan[*]	Nitzanim[*]	HaMa'apil[*]	Kishon	Kinneret	Beit She'an	Ein Feshkha	Ne'ot HaKikar[*]
Data set										
Molecular	13	10	15	10	2	10	16	13	13	15
Meristic	16	12	15	15	0	10	25	14	16	15
SL±sd (cm)	8.5±2.9	4.7±4.3	6.7±2	6.8±4.8		13.2±1.6	10.6±5.2	10.1±2.1	12.3±4.3	4.6±1.4
TR3±sd	6±0.5	6±0.7	6.1±0.3	6.2±0.7		7±0.6	6±0.4	6±0.8	7±0	5±0.6
LPR±sd	4±0	5±0	4±0	4±0		5±0.3	4.2±0.4	3.9±0.3	4±0	4±0.5

* Introduced population, SL = mean standard body length, sd = standard deviation of standard body length. HaMa'apil sample was not measured due to small sample size. TR3 = Number of scales between the lower lateral line and the anal fin. LPR = The position of the longest pectoral ray.

and more variable markers would allow a better reso-lution of the issues presented here, along with larger sample sizes per location.

Availability of supporting data

The data sets supporting the results of this article are available in GenBank under accession numbers EU163705-EU163723 and FJ613474-FJ613479 and in the Dryad Repository: http://dx.doi.org/10.5061/dryad.29g768sc.

Methods

Fish sampling and preparation

The research was approved by the Israel Nature and Parks Authority (permits 2006/25631, 2007/28858, 2008/31863). Fish were sampled using a seine net. Seven habitats were sampled: a) two coastal system habitats (Ein Afek on the Na'aman River, one sampling site; and the Taninim River, two sampling sites), b) the Kishon River (one sampling site), c) two habitats of the Jordan River system (Lake Kinneret, two sampling sites; and the Beit She'an Valley springs, three sampling sites), d) two Dead Sea system habitats (the Ein Feshkha Nature Reserve, two sampling sites; and the Ne'ot HaKikar Salt Flat, one sampling site). The sampling sites of each water body were at most five kilometers apart. Due to the small sample sizes, data from the same water body were pooled (i.e., they were treated as a single population) in the statistical analyses (AMOVA - Analysis of Molecular Variance and MANOVA - Multivariate Analysis of Variance). Consequently, our design did not allow us to analyze population structure within water bodies, although we cannot exclude the possibility that such a structure exists. We test here only the existence of structure among water basins.

Two artificial recreational ponds were also sampled (the Ramat-Gan park pond and the Nitzanim Nature Reserve pond, Figure 1). These artificial ponds are in the coastal basin. In addition, we obtained two *T. zillii* individuals from the HaMa'apil fish breeding farm. Unfortunately, other breeding farms did not allow us to examine their fish stock. For the morphological analysis, we also included specimens from the Steinhardt National Collection of Natural History, Zoological Museum at Tel-Aviv University (hereafter NCNH). Sample sizes and mean standard body lengths are provided in Table 4.

A liver sample was removed from each specimen collected (Table 4) and fixed in 70% ethanol for DNA extraction. The fish bodies were also preserved in 70% ethanol for meristic counts and submitted to the NCNH. While the individuals sampled were used for both morphological and genetic analysis, we extended our morphological sample size with the addition of fish from the NCNH. Unfortunately, the museum specimens were found to be unsuitable for DNA extraction. Before including these data in our morphological analysis, we

verified that the date of capture and methods of conservation were not significant factors, using the non-parametric MANOVA test [16] as implemented in the software PAST [17]. Sample sizes are provided in Table 4. Further sampling would have potentially put the small studied populations at risk.

DNA extraction and amplification

For each individual, 20–40 mg of liver tissue preserved in ethanol was homogenized with 500 μl TNES-Urea buffer [18] and 12.5 μl of proteinase K for 3 hours at 37°C. Following homogenization, DNA was extracted using a standard phenol-chloroform protocol followed by ethanol–sodium acetate precipitation. We selected the mitochondrial control region (D – loop) as our molecular marker, as in other studies of cichlid populations [19-21]. The control region was amplified using the primers Ormt 449 up [22] and a newly designed primer mit-tRNA-phe (5'-AGGGYCYATCTTAACATCTTCAGTG-3'). Amplified fragments were directly sequenced (to avoid cloning artifact) on an ABI PRISM 3100 (Applied Biosystems) genetic analyzer. The fragments were sequenced on both strands. Additionally, each polymorphic site and indel was confirmed manually by looking at the corresponding region on the chromatogram. Haplotypes were submitted to GenBank under accession numbers EU163705-EU163723 and FJ613474-FJ613479. Sequences were aligned using ClustalX [23].The resulting alignment was then refined manually. The final alignment included 862 positions with two indels [Additional file 1].

Sequence data analysis

The software Arlequin 3.0 [24] was used to compute several diversity indices (Table 1): the haplotype diversity h [25], the nucleotide diversity π [25,26] and two estimators of θ, θ_s, and θ_π. θ is the effective size of the population multiplied by the mutation rate. The θ_s estimate of θ is based on the relationship between the number of segregating sites and the sample size [27], while θ_π is the mean number of pairwise differences in the sample [26]. Both estimators assume the infinite site model. Since the mutation rate for the *T. zillii* control region sequence in unknown, the θ estimators can only be used to infer the relative effective sizes of the populations and consequently the relative effect of drift. Arlequin 3.0 was also used to conduct an AMOVA and to compute fixation index estimates (Φ estimates) [28]. The total molecular variance was partitioned into four "aquatic systems" (i.e., coastal, Kishon, Jordan River and Dead Sea), "Populations within aquatic system" and "within population" components. The sample sizes did not allow the examination of population structure within water bodies. Two analyses were conducted. In the first, samples from artificial habitats (i.e., Ramat-Gan and Nitzanim) were considered as coastal, since these habitats

are situated within the coastal basin, and the Ne'ot HaKi-kar sample, which has been suspected of having an aqua-cultural origin, was considered to belong to the Dead Sea system. In the second, samples of populations, which were probably introduced (i.e., Ramat-Gan, Nitzanim and Ne'ot HaKikar) were excluded. The HaMa'apil sample was excluded from the AMOVA analysis due to its small size (n = 2). Pairwise Φ_{st} values were tested with an exact con-tingency test as implemented in Arlequin 3.0. When aquatic systems or populations were found to be signifi-cantly different, Nei's corrected average numbers of differ-ences D [29] were computed between systems or populations with the program Arlequin, using Jukes-Can-tor distances. Finally, a median-joining network [30] was reconstructed with the program Network 4.2.0.1 (available at http://www.fluxus-engineering.com). In all analyses, gaps were treated as a fifth character.

Meristic data acquisition and analysis

Twenty-two meristic characters were counted manually (Table 3). The data were logarithmically transformed to eliminate size-related differences [31]. The meristic counts were analysed using a nested MANOVA to examine whether the variation of morphological traits was signifi-cantly partitioned by geographical factors (i.e., among populations and among aquatic systems) discriminant ana-lysis was used to visualize the morphological variation among populations [32-34]. These analyses were con-ducted with the software Statistica (StatSoft) and PAST. Morphological data were deposited in the Dryad Reposi-tory: http://dx.doi.org/10.5061/dryad.29g768sc.

Additional file

Additional file 1: *Tilapia zillii* D-loop sequence alignment. Description: D-loop sequence alignment used in the analysis in Fasta format. TM, Taninim (coastal); EA, Ein Afek (coastal); KN, Kishon (Kishon); KT, Kinneret (Jordan); BS, Beit She'an (Jordan); EF, Ein Feshkha (Dead Sea); NH, Ne'ot HaKikar (Dead-Sea introduced); RG, Ramat Gan (coastal introduced); NM, Nitzanim (coastal introduced); MA, HaMa'apil (coastal introduced).

References
1. Chakrabarty P: Cichlid biogeography: comment and review. *Fish Fish* 2004, **5**:97–119.
2. Lesica P, Allendorf FW: When are peripheral populations valuable for conservation. *Conserv Biol* 1995, **9**:753–760.
3. Goren M, Ortal R: Biogeography, diversity and conservation of the inland water fish communities in Israel. *Biol Conserv* 1999, **89**:1–9.
4. Fishelson L, Bresler V: Comparative studies of the development and differentiation of chloride cells in tilapine fish with different reproductive styles. *J Morphol* 2002, **253**:118–131.
5. Briggs JC: Freshwater fishes and biogeography of Central America and the Antilles. *Sys Zool* 1984, **33**:428–435.
6. Stiassny MLJ: Phylogenetic intrarelationships of the family Cichlidae: an overview. In *Cichlid fishes—behaviour, ecology and evolution*. Edited by Keenleyside MHA. London: Chapman & Hall; 1991:1–35.
7. Bruton MN, Gophen M: The effect of environmental factors on the nesting and courtship behavior of *Tilapia zillii* in Lake Kinneret (Israel). *Hydrobiologia* 1992, **239**:171–178.
8. Snovsky Z, Shapiro G: *Fishing and Aquaculture in Israel in 2000*(in Hebrew). Israeli Ministry of Agriculture: Department of Fisheries; 2001.
9. Zimmels Y, Kirzhner F, Roitman S: Use of naturally growing aquatic plants for wastewater purification. *Water Environ Res* 2004, **76**:220–230.
10. Por FD, Dimentman C: *The Legacy of Tethys - an Aquatic Biogeography of the Levant*. Dordrecht: Kluwer Academic Publishers; 1989.
11. Arndt A, Van Neer W, Hellemans B, Rubben J, Volckaert F, Waelkens M: Roman trade relationships at Sagalassos (Turkey) elucidated by ancient DNA of fish remains. *J Archaeological Sci* 2003, **30**:1095–1105.
12. Hickling CF: *Fish Culture*. 2nd edition. London: Faber and Faber; 1971.
13. Vermeersch PM, Paulissen E, Vanpeer P: The Paleolithic of the Egyptian Nile Valley. *Anthropologie* 1990, **94**:435–458.
14. Sarig S: A review on *Tilapia* culture in Israel. In *Proceedings of the International Symposium on Tilapias in Aquaculture*. Edited by Fishelson L, Yaron Z. Tel Aviv, Israel: Tel Aviv University; 1983.
15. Loya Y, Fishelson L: Ecology of fish breeding in brackish water ponds near the Dead Sea (Israel). *J Fish Biol* 1969, **1**:261–278.
16. Anderson MJ: A new method for non-parametric multivariate analysis of variance. *Aust Ecol* 2001, **26**:32–46.
17. Hammer O, Harper DAT, Ryan PD: PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electron* 2001, **4**. art. 4.
18. Asahida T, Kobayashi T, Saitoh K, Nakayama I: Tissue preservation and total DNA extraction from fish stored at ambient temperature using buffers containing high concentration of urea. *Fish Sci* 1996, **62**:727–730.
19. Bowers N, Stauffer JR, Kocher TD: Intra and interspecific mitochondrial DNA sequence variation within two species of rock-dwelling cichlids (Teleostei: Cichlidae) from Lake Malawi, Africa. *Mol Phylogenet Evol* 1994, **3**:75–82.
20. Sturmbauer C, Baric S, Salzburger W, Ruber L, Verheyen E: Lake level fluctuations synchronize genetic divergences of cichlid fishes in African lakes. *Mol Biol Ecol* 2001, **18**:144–154.
21. Verheyen E, Ruber L, Snoeks J, Meyer A: Mitochondrial phylogeography of rock-dwelling cichlid fishes reveals evolutionary influence of historical lake level fluctuations of Lake Tanganyika, Africa. *Philos Trans R Soc Lond Ser B Biol Sci* 1996, **351**:797–805.
22. Nagl S, Tichy H, Mayer WE, Samonte IE, McAndrew BJ, Klein J: Classification and phylogenetic relationships of African tilapiine fishes inferred from mitochondrial DNA sequences. *Mol Phylogenet Evol* 2001, **20**:361–374.
23. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG: The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997, **25**:4876–4882.
24. Excoffier LG, Schneider S: Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol Bioinform Online* 2005, **1**:47–50.
25. Nei M: *Molecular Evolutionary Genetics*. New York: Columbia University Press; 1987.
26. Tajima F: Evolutionary relationship of DNA sequences in finite populations. *Genetics* 1983, **105**:437–460.
27. Tajima F: Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 1989, **123**:585–595.

28. Excoffier L, Smouse PE, Quattro JM: **Analysis of molecular variance infered from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data.** *Genetics* 1992, **131**:479–491.
29. Nei M, Li WH: **Mathematical model for studying genetic variation in terms of restriction endonucleazes.** *Proc Natl Acad Sci USA* 1979, **76**:5269–5273.
30. Bandelt HJ, Forster P, Rohl A: **Median-joining networks for inferring intraspecific phylogenies.** *Mol Biol Evol* 1999, **16**:37–48.
31. Barriga-Sosa IDLA, Jimenez-Badillo MDL, Ibanez AL, Arredondo-Figueroa JL: **Variability of tilapias (***Oreochromis* **spp.) introduced in Mexico: morphometric, meristic and genetic characters.** *J Appl Ichthyol* 2004, **20**:7–14.
32. Adams DC, Rohlf FJ, Slice DE: **Geometric morphometrics: ten years of progress following the 'revolution'.** *Ital J Zool* 2004, **71**:5–16.
33. Hammer Ø: *Morphometrics - Brief Notes*. Zürich: Paläontologisches Institut und Museum; 2002.
34. Marcus LF: **Some aspects of multivariate statistics for morphometrics**. In *Contributions to Morphometrics*. Edited by Marcus LF, Bello E, Garcia-Valdecasas A. Madrid: Monographias del Museo Nacional de Ciencias Naturales; 1993:95–130.

Submit your next manuscript to BioMed Central and take full advantage of:

- **Convenient online submission**
- **Thorough peer review**
- **No space constraints or color figure charges**
- **Immediate publication on acceptance**
- **Inclusion in PubMed, CAS, Scopus and Google Scholar**
- **Research which is freely available for redistribution**

Submit your manuscript at
www.biomedcentral.com/submit

BioMed Central