Invasion Biology Meets Parasitology: A Case Study of Parasite Spill-Back with Egyptian *Fasciola gigantica* in the Invasive Snail *Pseudosuccinea columella*

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Abstract

The liver fluke *Fasciola gigantica* is a trematode parasite of ruminants and humans that occurs naturally in Africa and Asia. Cases of human fascioliasis, attributable at least in part to *F. gigantica*, are significantly increasing in the last decades. The introduced snail species *Galba truncatula* was already identified to be an important intermediate host for this parasite and the efficient invader *Pseudosuccinea columella* is another suspect in this case. Therefore, we investigated snails collected in irrigation canals in Fayoum governorate in Egypt for prevalence of trematodes with focus on *P. columella* and its role for the transmission of *F. gigantica*. Species were identified morphologically and by partial sequencing of the cytochrome oxidase subunit I gene (COI). Among all 689 snails found at the 21 sampling sites, *P. columella* was the most abundant snail with 296 individuals (42.96%) and it was also the most dominant species at 10 sites. It was not found at 8 sites. Molecular detection by PCR and sequencing of the ITS1-5.8S-ITS2 region of the ribosomal DNA (rDNA) revealed infections with *F. gigantica* (3.38%), *Echinostoma caproni* (2.36%) and another echnostome (7.09%) that could not be identified further according to its sequence. No dependency of snail size and trematode infection was found. Both high abundance of *P. columella* in the Fayoum irrigation system and common infection with *F. gigantica* might be a case of parasite spill-back (increased prevalence in final local hosts due to highly susceptible introduced intermediate host species) from the introduced *P. columella* to the human population, explaining at least partly the observed increase of reported fascioliasis-cases in Egypt. *Eichornia crassipes*, the invasive water hyacinth, which covers huge areas of the irrigation canals, offers safe refuges for the amphibious *P. columella* during molluscicide application. As a consequence, this snail dominates snail communities and efficiently transmits *F. gigantica*.

Introduction

The liver fluke *Fasciola gigantica* is a trematode parasite native to Africa and Asia and infects ruminants, but also humans as final hosts. Adult *F. gigantica* live in the bile ducts of the liver, where they can reach a length of up to 76 mm. Infection with this parasite can cause severe disease symptoms referred to as fascioliasis [1]. Intermediate hosts are various lymnaeid snails [2], inside of which the parasite proliferates asexually and produces free swimming cercariae that will attach to submerged surfaces, mostly plants. These cercariae develop into encysted and durable metacercariae which are transmitted when the final host ingests the metacercariae together with plants, or by consumption of water contaminated with metacercariae [3]. Due to the durability of the metacercariae, transmission can also be mediated by ingestion of terrestrial plants and crops that were submerged in water containing infected snails for a couple of weeks [1,4], which is a common irrigation technique in Fayoum area. Especially in Egypt, fascioliasis is an increasing problem, reaching prevalences in animals of sometimes more than 50% and up to 19% in humans [5–7]. According to estimations of the World Health Organization [8], at least 830,000 people are infected with either the introduced *Fasciola hepatica* or *F. gigantica* in the Nile delta. Presence of both of these closely related species has been confirmed for Egypt [1,9,10], but they are usually not distinguished diagnostically [11].

The natural first intermediate host of *F. gigantica* in Egypt is the snail *Radix natans*, but the trematode was also found commonly in the introduced species *Galba truncatula* [12]. Additionally, single cases of *F. gigantica* infections were reported from *Biomphalaria alexandrina* and *Pseudosuccinea columella* in Egypt [13–15]. For the latter species, release of *F. gigantica* cercariae was proven in laboratory infections [16]. The highly invasive lymnaeid snail *P. columella* was introduced from North America to many countries worldwide and was reported from Africa for the first time in the
middle of the 20th century, where it is now widely distributed [17–
20]. *P. columella* is well known as a suitable host for *F. hepatica* [12],
but its importance for the maintenance of the natural life cycle of
*F. gigantica* and the transmission to humans is uncertain.

According to the parasite spill back hypothesis, invasive species
are colonized by local parasites. If the invader becomes abundant
and the parasite can develop successfully, a high number of
transmitting stages will develop and increase local parasite
abundance and prevalence [21]. In this way, free living invasive
species may help native parasites to increase their population size
and extend their distribution range [22]. *P. columella* is a very
efficient invader and became an important snail host for *F. hepatica*
in many countries [12]. We hypothesize that this might be the case
for the *P. columella*/*F. gigantica*—system as well. As a result, infected
*P. columella* might be responsible for the observed increase in
infection intensity and prevalence in livestock and in the human
population in Egypt. Therefore, we investigated the trematode
species occurring in the invasive snail *P. columella*, collected from
irrigation channels in the Fayoum governorate where cases of
fasciolasis are commonly reported [23,24]. We assessed the
potential of this invasive species as a host for trematodes, especially
for *Fasciola* spp. to estimate the effect of *P. columella* for the spread
of fasciolasis in the area and found evidence for a possible spill-
back effect on animals and the human population.

**Materials and Methods**

**Snail sampling**

Snails were collected from July to September 2012, 2 month
after the last molluscicide treatment, from water plants (mainly
water hyacinths *Eichhornia crassipes*) and with dip nets in irrigation
channels at 21 different sites in Fayoum governorate (surrounding
Markaz El-Fayoum, Isla, and Ibsway cities), Egypt. Samples were
taken with permission of the local farmers owning the land
adjacent to the irrigation channels. No endangered or protected
species were sampled. Sampled snails were fixed in 99% ethanol
for molecular analysis. Collected specimens from the different
sampling sites were identified morphologically according to the
key of Brown [19] and by molecular biology. After identification,
*P. columella* individuals were measured (shell length) and crushed
to check visually for trematode infections. Samples of the soft tissue
(for molecular analysis of parasites) and the foot muscle (for
molecular species identification of all snails; presumed to be free of
parasites) were taken and frozen at −20°C for molecular analyses.

**Molecular analyses**

Snail tissue samples were homogenized in 1.5 ml reaction tubes
with micropestles (Eppendorf) and DNA was extracted with a
JETQUICK DNA Clean-Up Spin Kit (Genomed) according to
manufacturer’s instructions. Molecular species identification of the
snails was done by sequencing of the Folmer-region of the
cytochrome oxidase subunit 1 (*COI*) with the primers LCO1490
and HC02198 [25] (about 700 bp). At least two individuals of each
species were sequenced to confirm morphological identification.

For molecular detection of trematode infections in the soft tissue
of *P. columella*, the universal trematode primers *Trem*1 F/*Trem*1 R
were designed that amplify a short part of the internal transcribed
spacer 2 (ITS2) and the beginning of the 28S ribosomal DNA
(rDNA) (about 200 bp). For sequencing, an additional PCR with
the primers *Trem*2 F (end of 18S rDNA) and *Trem*1 R
was conducted to obtain a longer sequence, including almost the whole
internal transcribed spacer 1 (ITS1) – 5.8S rDNA– ITS2 region of
the ribosomal DNA (about 1300 bp). The primer pair *Fasc*-
ITS1 F/R was designed for specific amplification of a 716 bp
ITS1-segment of both *F. gigantica* and *F. hepatica*, to distinguish
*Fasciola* spp. from host and infections with other trematodes.

Sequences and additional information for primers designed for the
present study are given in table 1. One 20 μl PCR reaction mix
contained 4 μl of 3× Crimson Taq buffer (New England Biolabs),
0.2 mM dTTP mix (New England Biolabs), 0.5 μM of each
primer 0.5 U Crimson Taq (New England Biolabs) and 1 μl
template DNA. The mix was topped up to 20 μl with PCR grade
water. The DNA was amplified by a Labcyber (SensoryQuest)
under the following conditions: initial denaturation at 95°C for
5 min, 40 cycles of 95°C for 30 s, annealing (temperatures see
table 1) for 30 s and elongation at 72°C for 30 s followed by a final
elongation of 72°C for 5 min.

Sensitivity of the *Trem*2 F/*Trem*1 R primer pair was lower and
not all samples were amplified successfully with the Crimson Taq,
although tested positive before with *Trem*1 F/*Trem*1 R primers.
Therefore, the more robust Phire Animal Tissue Direct PCR Kit
(Thermo Scientific) was used for amplification with those primers.
Reactions contained 10 μl of 2× Phire PCR Buffer, 0.5 μM of
each primer, 0.4 μl Phire Hot Start II DNA Polymerase and 1 μl
DNA. Water was added to 20 μl. PCR conditions were 98°C for
5 min, 40 cycles of 98°C for 10 s, annealing at 54°C for 10 s,
elongation at 72°C for 20 s and a final elongation at 72°C for
1 min. PCR conditions for the LCO1490/HC02198 primers were
as described in Folmer et al. [25]. PCR products were checked by
standard agarose gel electrophoresis, purified with a JETQUICK
PCR Product Purification Spin Kit (Genomed) according to
manufacturer’s instructions and sent for sequencing (GATC).
Sequences were checked for homology with database entries by
BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Statistics**

Mean prevalences and confidence intervals of parasite infections
were calculated with the program Quantitative Parasitology v.3.0
[26]. Dependency of snail size and infection was analyzed by
logistic regression using R v.3.0.1 [27].

**Results**

**Snails**

In total, 9 different snail species were identified morphologically
and genetically. The sequences of different isolates of each species
were identical, so one representative *COI*—sequence of each species
was deposited in GenBank. Sorted by their abundance, the
following species were found: *Pseudosuccinea columella* (accession
no. KF412765), *Physa heterostropha* (accession no. KF412768),
*Cleopatra bulimoides* (accession no. KF412769), *Bulimus truncatus*
(accession no. KF412767), *Melanoides tuberculata* (accession
no. KF412770), *Biomphalaria alexandrina* (accession no. KF412766),
*Succinea sp.* (accession no. KF412772), *Bellamya sp.* (accession
no. KF412773) and *Theodoxus anatolicus* (accession no. KF412771). The five
*COI* sequences obtained from *P. columella* individuals were
between 97%–100% similar to the different isolates of *P. columella*
in GenBank.

Numbers of snails and species diversity varied greatly between
sampling sites. The highest number of snails was found at Disya
Zawyet El-Karadsah-site with 6 different snail species, while only a
single snail species was found at two sites (*P. heterostropha* in El-Girb
and *C. bulimoides* in Qalamshah village). *P. columella* was not
only the most abundant snail in total numbers (296 of 689,
42.96%; see fig. 1), but it was also the most dominant species at 10
The second and third most abundant snails were \textit{P}. \textit{heterostropha} (19.59\%) and \textit{C}. \textit{bulimoides} (9.43\%), respectively (fig. 1). The latter species was found mostly in the locality of Izbat Ashur and Hawwarat Al-Maqa. Proportions of the other snails found in the area were 8.56\% for \textit{B}. \textit{truncatus}, 6.53\% for \textit{M}. \textit{tuberculata}, 4.35\% for \textit{B}. \textit{alexandrina}, 3.92\% for \textit{Succinea} sp., 2.76\% for \textit{Bellamya} sp. and 1.89\% for \textit{Theodoxus anatolicus}. Detailed results on the number of snails and snail species at each site are shown in table 2. Size range of \textit{P}. \textit{columella} individuals varied between 0.15 and 1.18 cm. Mean and median size were 0.54 and 0.50 cm, respectively.

**Trematodes**

No trematode stages were found in \textit{P}. \textit{columella} specimens by visual inspection, as ethanol fixation made differentiation between host and parasite tissue impossible. By PCR with the Trem1 F/ Trem1 R primers, 38 of the 296 \textit{P}. \textit{columella} specimens collected in total at all sampling sites were positive for trematode infection (12.84\% [confidence interval (CI) 9.42–17.19\%]). Sequencing of the ITS1-5.8S-ITS2 rDNA regions (with the Trem2 F/Trem1 R primers) revealed that 7 (2.36\% [CI 1.12–4.83\%]) of those snails were infected with \textit{Echinostoma caproni} (accession no. KF425322) according to 98\% sequence identity with AJ564382 (isolate from Cairo). \textit{F. gigantica} was detected in 10 \textit{P}. \textit{columella} individuals (3.38\% [CI 1.81–6.18\%]) by PCR with Fasc ITS1 F/R primers and sequencing with Trem2 F/Trem1 R (accession no. KF425321; distinguished from \textit{F. hepatica} according to variable positions listed in Mas-Coma et al. 2009). An infection with an unknown echinostome trematode was found in 21 \textit{P}. \textit{columella} individuals (7.09\% [CI 4.60–10.60\%]). The closest match for this sequence in the GenBank was the echinostome \textit{Philophthalmus lucipetus} with only 85\% similarity, therefore a more detailed identification was not possible. Figure 2 illustrates the overall prevalences of the three trematodes found. The highest prevalence for trematode infections was found in \textit{P. columella} from El-Misharrak site, where 9 of 26 snails (34.62\%) were infected, while the lowest infection rate was detected in Itsa (Bahr Arus) with one infected snail among 18 (5.56\%). \textit{E. caproni} was found at three and the unknown echinostomid at 8 out of 10 sites. \textit{F. gigantica} infected snails were present at 5 of the 21 sampling sites with a prevalence of up to 11.11\% (Al Hadeer village), but with only one infected snail out of nine. The lowest prevalence of \textit{F. gigantica} was detected in Sayyidna Al-Khidr village with 4.76\% (2 out of 42 \textit{P}. \textit{columella}). At three sites, no trematode infection was detected in \textit{P. columella}, but in these cases snail numbers were low (8 in Tutun village, 2 in Izbat Ezbat El-Eslah El-Zraei and 1 in Zawyet El-Karadsah

| Name  | Sequence | Target region | Annealing Temp. | Approximate length of product |
|-------|----------|---------------|-----------------|------------------------------|
| Trem1 F | TAG CCT YGG ATC AGW CGT GA | ITS2 | 54°C | 200 bp with Trem1 R |
| Trem2 F | CAA GTC ATA AGC TGC TGA | 185 rDNA | 54°C | 1300 bp with Trem1 R |
| Trem1 R | ACC YAA ACA CCA CAT TGC CTA | 285 rDNA | 54°C | |
| Fasc ITS1 F | TCT ACT CTT ACA CAA GGG ATG CAC | ITS1 | 55°C | 716 bp |
| Fasc ITS1 R | GGC TTT CTG CCA AGA CAA G | |

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![Figure 1. Total numbers of the snail species found at all sites.](doi:10.1371/journal.pone.0088537.g001)
| Sampling sites       | Disya village | Isra (Bahr El'Ghaba) | Izbat Ashur | Zawyet El'Elmar | Hamzeit Al'Harf | Shbya Al'Harf village | Izbat El'Bank | Ahmed Al'Bari village | Al'Arbi village | Tutun village | El'Harun village | Abu Elba | Isra (Bahr Arus) | Izbat Hamada Dahman | Al'Harun village | El'Aramia Isra village | Qalamshah village | El'Atamna Isra village | El'Kharag village | El'Harun village | Hanna Habib village | Qalamshah village | El'Kharag village |
|----------------------|---------------|----------------------|-------------|---------------|----------------|-----------------------|--------------|------------------------|----------------|--------------|-------------------|----------|----------------|------------------------|-------------------|------------------------|------------------|-------------------|-------------------|------------------|------------------|------------------|
| Sampling sites       | 68            | 56                   | 1           | 42            | 14             | 14                    | 2            | 24                     | 14             | 8            | 5                 | 7                    | 18              | 16                 | 8                    | 5                 | 2                        | 5                | 2                    | 2              | 1                | 2                | 2                | 1                |
| No. of individuals   | 8             | 8                    | 10          | 11            | 2              | 4                     | 4            | 2                      | 4              | 1            | 1                 | 7                    | 14              | 18                 | 13                   | 2                 | 2                        | 1                | 2                    | 1                | 2                | 1                | 2                | 1                |
| No. of species       | 9             | 6                    | 8           | 10            | 2              | 4                     | 1            | 4                      | 2              | 1            | 1                 | 4                    | 14              | 3                  | 13                   | 2                 | 2                        | 1                | 2                    | 1                | 2                | 1                | 2                | 1                |
| Proportion of total no. | 42.96%  | 19.59%               | 9.43%       | 8.56%         | 6.53%          | 4.35%                 | 3.92%        | 2.76%                  | 1.89%          | 1.89%        | 1.89%             | 9.43%               | 8.56%           | 6.53%              | 4.35%                | 3.92%             | 2.76%                     | 1.89%            | 1.89%               | 1.89%            | 1.89%           | 1.89%            | 1.89%           | 1.89%          |
Discussion

The results of the present study show that the invasive snail *P. columella* is found frequently in irrigation channels in the Fayoum governorate (at 61.90% of sites investigated in the present study) and it even turned out to be the most abundant snail species at most sites. Additionally, our findings imply that *P. columella* probably became one of the major snail intermediate host species for *F. gigantica*. Although *F. gigantica* infections in *P. columella* have been reported before [14], this is the first study to provide molecular data for this host-parasite relationship. Possibly, the spread of *P. columella* as an additional intermediate host explains partly the increase of human fascioliasis cases in Egypt in the last 50 years [28], as well as the occurrence of hyperendemic outbreaks in the Nile delta [3,6]. Both *P. columella* species have been identified from parts of Egypt [9,29,30], but until now, no information was available for Fayoum governorate. Only *F. gigantica* was found in the irrigation system of the Fayoum oasis in the present study, indicating that this parasite is the major cause of fascioliasis cases reported in humans [23] and animals [7,24] in that area. The host snail, *P. columella*, is well known to be a suitable host for *F. hepatica* [12], but apparently it can maintain the life cycle of *F. gigantica* as well. Surprisingly, *Radix natalensis*, the indigenous host for *F. gigantica* was not found at all within the present study. This might be explained by seasonal peaks in abundance of the two species reported by Ahmed & Ramzy [14]. These authors observed that *P. columella* was predominant in autumn and *R. natalensis* from December to February. If this is the case in the area investigated in the present study, it might come to an increased infection pressure due to the presence of infected intermediate hosts throughout the year. Another reason for the absence of *R. natalensis* in the samples of the present study might also be the high requirements of this species on water quality and oxygen levels [14,31] that can be limiting in the eutrophic irrigation channels, especially at high temperature. Compared to *P. columella*, *R. natalensis* might also be more sensitive to the molluscicide treatment in the channels, which gives the invader an additional selective advantage. In Brazil, *P. columella* was reported to be the only available host snail in some areas where *F. hepatica*-infections were reported [12], therefore this snail might also be able to maintain the life cycle of *F. gigantica* in Egypt. During a parasitological examination of lymnaid snails in Dakahlia governorate (sampling date was not reported), El-Shazly et al. [32] found mainly *R. natalensis* (68.4%) as well as *G. truncatula* (16.0%), but only few *P. columella* (3.4%). Also, no infection with *Fasciola* sp. was detected in *P. columella* in their study, indicating that *F. gigantica* is transmitted mainly by the natural intermediate host if it is present. According to previous experimental studies, *P. columella* is less susceptible to Cuban *F. hepatica* and produced lower numbers of rediae than the indigenous *Galba Cabrerae* [33]. This indicates that *P. columella* might not be a relevant host for *F. hepatica* (and possibly *F. gigantica* as well), if the natural snail host is present.

### Table 3. Number of infected *P. columella* and prevalences for each trematode and site (total no. of tested snails given in brackets).

| Site                  | Fasciola gigantica | Echinostoma caproni | unknown Echinostomoid | all trematodes |
|-----------------------|--------------------|---------------------|-----------------------|---------------|
| El-Misharrak village  | 2 (26)/7.69%       | 2 (26)/7.69%        | 9 (26)/34.62%         |               |
| Sayidna Al-Khidr village | 2 (42)/4.76%  | 3 (42)/7.14%        | 7 (42)/16.67%         |               |
| Itsa (Bahr El Ghaba) | 3 (68)/4.41%       | 7 (68)/10.29%       | 10 (68)/14.71%        |               |
| El-Khawagat village  | 2 (14)/14.29%      | 2 (14)/14.29%       |                      |               |
| Al Hadeer village    | 1 (9)/11.11%       | 1 (9)/11.11%        |                      |               |
| Ahmed Afandi village | 2 (24)/8.33%       | 2 (24)/8.33%        |                      |               |
| Al Amiriyah village  | 3 (56)/5.36%       | 1 (56)/7.14%        | 4 (56)/7.14%          |               |
| Abu Ish              | 1 (14)/7.14%       | 1 (14)/7.14%        |                      |               |
| Izbat El-Bank        | 1 (14)/7.14%       | 1 (14)/7.14%        |                      |               |
| Itsa (Bahr Arus)     | 1 (18)/5.56%       | 1 (18)/5.56%        |                      |               |
| Total no. and mean prevalence with 95% CI | 10 (296)/3.38% [1.81–6.18%] | 7 (296)/2.36% [1.12–4.83%] | 38 (296)/12.84% [9.42–17.19%] |   |

Results sorted in decreasing order by prevalence of all trematodes.

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Several studies reported a rather high prevalence of liver flukes in their snail hosts. Caron et al. [34] found 6.25% of G. truncatula infected with Fasciola sp. in Algeria. In Dakahlia governorate, similar prevalences were reported in R. natatoris (5.30%) and G. truncatula (3.10%) [32]. Prevalences of F. gigantica observed in the present study (mean 3.83%) were within the range that was reported from P. columella in a previous study in Giza governorate [14], although F. gigantica infected snails in the present study were smaller (average of 0.40 cm) than reported by Ahmed & Ramzy [14], who noticed that most infected snails were larger than 1 cm. This might be due to the generally rather small size of snails in the present study with only a few individuals larger than 1 cm. Apparently, the infection is not restricted to large snails and can also occur in populations where only smaller snails are present.

One important factor that might lead to increased abundance of P. columella, but also other amphibious snails, is the water hyacinth (Eichhornia crassipes) that is present in most surface waters in Egypt. Like P. columella, E. crassipes is an invasive species in Egypt. It was introduced in Africa by the end of the 19th century and spread throughout the continent [35]. According to recent estimations, the total area infested with water hyacinths in Egypt is as large as 487 km², covering large parts of irrigation channels all over the country [36]. The resulting problematic link of aquatic vegetation, snail abundance and increased infection rates with Schistosoma spp. and Fasciola spp. was already recognized long ago and has been further studied since then, mainly with focus on vectors of schistosomiasis [37,38]. Depending on focal outbreaks of diseases, the irrigation channels in Fayoum governorate are treated in loose networks of irrigation channels that provide ideal habitat for vector molluscicides by the Ministry of Health, especially for prevention of schistosomiasis. In this context, water hyacinths will not only provide a habitat for P. columella, but might also be a refuge for the snail, when molluscicides are present in the water. Amphibious snails like P. columella might just move on the plants above water level and endure until the waves of molluscicides have passed. P. columella can also withstand detrimental circumstances by digging into moist mud where the snails survive even centimeters away from water [16]. Their ability to survive adverse conditions might be one reason for the success of this snail as an invader and explains the dominance of P. columella at most sampling sites in the present study.

We believe that the reported scenario of invasive P. columella as efficient snail host for F. gigantica represents a case of parasite spill-back, resulting in a drastic increase of infections in humans. This situation might be aggravated by the presence of water hyacinths, another invader that provides habitat for the snail intermediate host, altogether giving a good example how invasive species can alter biotic conditions and influence parasite life cycles, in this case of a human pathogen. A similar situation is likely to occur in many other agricultural areas in Africa.

Besides F. gigantica, E. caproni and an unidentified echinostome were also detected in the snails tested in the present study. To the best of our knowledge, this is the first report of E. caproni in P. columella. Echinostome cercariae are released from the first intermediate host snail, infect other snails and use them as second intermediate host where they form metacercariae. The respective final host becomes infected by ingestion of snails containing metacercariae. Detection of trematodes by molecular methods in the present study does not allow for distinction of sporocysts/rediae and metacercariae in the snails, but most likely P. columella is used as second intermediate host for E. caproni that normally infects Biomphalaria spp. as first intermediate hosts [39]. In cases of co-infections with Schistosoma mansoni and echinostome trematodes in the same snail, the latter were found to impair infection, development and infectivity of schistosome cercariae or even to consume larvae of other trematodes in the same snail [reviewed by Fried & Huffman and Loker & Adema [39,40]]. In case P. columella would prove to be first intermediate host for echinostomes, these parasites might have to be considered as regulating factor for F. gigantica. Therefore, the presence of echinostomes in P. columella in the Fayoum irrigation system might reduce the level of F. gigantica-infections in the area.

A recent study revealed the presence of different morphologically indistinguishable lineages F. gigantica from Africa and India that might in fact be two separate species, as well as an African highland lineage of F. hepatica-like flukes that use Gaira truncatula as snail host and can be clearly differentiated genetically from European F. hepatica [41]. Various other studies have shown that species boundaries seem to be not clear for F. hepatica and F. gigantica (see review in Mas-Coma et al. [42]). Also, intermediate Fasciola individuals were described from Iran and Egypt, sharing characteristics of F. hepatica and F. gigantica [30,43,44]. The ITS1-5.8S-ITS2 rDNA sequences of F. gigantica from isolates obtained in the present study did not show the polymorphic sites characteristic for the intermediate forms reported by Amer et al. [44]. Therefore, we consider the infection in P. columella as "regular" F. gigantica. Nevertheless it is of great importance to clarify the identity of intermediate forms as well as lineages of F. gigantica and F. hepatica, their host spectrum and significance for human infections.

The large Fayoum oasis is a fertile agricultural area with a tight network of irrigation channels that provide ideal habitat for vector snails especially for Schistosoma and Fasciola spp. The invasive snail P. columella will contribute to the increased prevalence of fascioliasis in the human population in that area and put the population of about 2.9 million people [http://www.geohive.com/] at risk. Many of them live in agricultural areas and are exposed to parasitological problems linked to the irrigation system directly, but fascioliasis, in contrast to schistosomiasis, might even affect the urban populations, if crops bearing metacercariae are transported to the cities [1]. The present study showed that F. gigantica seems to be the major cause of fascioliasis in the Fayoum governorate and that the invasive snail P. columella is responsible for the maintenance of the infection in spite of snail eradication programs. Further studies are required to evaluate the effectiveness of molluscicide treatments on P. columella and to evaluate alternative approaches of snail control.

Author Contributions
Conceived and designed the experiments: DSG FAMMM MN EMHM AHAS BS. Wrote the paper: DSG.

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