Human-aided dispersal facilitates parasitism escape in the most invasive mosquito species

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Abstract.

Human-aided invasion of alien species across the world sometimes leads to economic, health or environmental burdens. During invasion process, species encounter new environments and partially escape some ecological constrains they faced in their native range, while they face new ones. The Asian tiger mosquito *Aedes albopictus* is one of the most iconic invasive species that was introduced in every inhabited continent over a short period of time due to international trade. It has also been shown to be infected by a prevalent and yet disregarded gregarine entomoparasite *Ascogregarina taiwanensis*. In this study, we aimed at deciphering the global dynamics of *As. taiwanensis* infection in natural *Ae. albopictus* populations and we further explored factors shaping its distribution. We showed that *Ae. albopictus* populations are highly colonized by several *As. taiwanesis* genotypes but recently introduced ones are escaping the parasite. We further performed experiments to explain such pattern. First, we hypothesized that founder effects (*i.e.* population establishment by a small number of individuals) may influence the parasite dynamics. This was confirmed since experimental increase in mosquitoes' density improves the parasite horizontal transmission to larvae. Furthermore, *Ae. albopictus* larvae do not exhibit density dependent prophylaxis to control the parasite meaning that infection is not mitigated when larval density increases. Secondly, we hypothesized that unparasitized mosquitoes were more prompt to found new populations through active flight dispersal. This was, however, unlikely since parasitized mosquitoes tend to be more active than their unparasitized relatives. Finally, we hypothesized that mosquito passive dispersal (*i.e.* often mediated by human-aided transportation of dried eggs) affects the parasite infectiveness. Our results support this hypothesis since parasite infection decreases over time when dry eggs are stored. This study highlights the importance of global trade on parasitism escape in one of the most invasive vector species on earth.

Introduction.

Despite repeated calls on actions to prevent drastic consequences of global change, our society is now facing many ecological challenges that could profoundly affect its future (Atwoli et al., 2021; Pautasso et al., 2015; Ricciardi et al., 2019). Ecological niches are currently driven to more drastic conditions for biodiversity. The cumulative effects of invasive species and human activities are a threat to biodiversity due to change in global species distributions but also species loss and drift in community composition related to parasite transmission or competitive exclusion (Bellard et al., 2016; Bradshaw et al., 2016; Decaëns et al., 2018; Didham et al., 2005; Doherty et al., 2016; Mainka & Howard, 2010). Introduced species tend to raise health, agricultural, goods and services costs that weaken the global economy (Andersen et al., 2004; Bradshaw et al., 2016; Cuthbert et al., 2021; Diagne et al., 2021; Olson, 2006). Finally, some invasive species are considered as a threat to human health due to their pathogenicity or their ability to transmit pathogens (Hulme, 2014). For example, the invasive raccoon *Procyon lotor* combines all adverse aspects listed above since it degrades agricultural landscapes, induces biodiversity decrease (Suzuki & Ikeda, 2020) and it is a vector of *Baylisascaris procyonis*, a nematode responsible for animal and human diseases (Duscher et al., 2021). Benefits of invasive species for biodiversity, economy and health should not be neglected but those are often difficult to estimate and poorly reported (Chapman, 2016). For instance, the prickly pear *Opuntia ficus-indica* is an invasive plant from arid and semi-arid regions (El-Mostafa et al., 2014) that serves as an animal food source and contains antioxidant, antimicrobial or anti-inflammatory compounds (M. A. Silva et al., 2021). Currently, 37% of the 66 most-studied invasive species are clearly considered as a threat while uncertainties remain on others (Shackleton et al., 2019). The main characteristic conditioning invasion success is the propagule (or colonization) pressure that is related to the quantity and frequency of non-native species colonizing a new area (Lockwood et al., 2009). This process requires enough individuals to pass a series of filters such as transportation survival, local dispersal, and population establishment leading to invasion success (Colautti & MacIsaac, 2004). Traits often enable successful invasive species to be acclimated in new ecosystems, to increase in population densities sometimes faster than native species. Consequently, reproduction and survival abilities in the focal ecosystem are particularly important for invaders. For instance, foreign fishes in southeastern Pyrenees watershed have shown a higher fecundity associated to equivalent water quality flexibility in comparison to native species (Vila-Gispert et al., 2005). Other traits such as increase in longevity and body size differentiate foreign fishes from native ones. Similar traits were also reported for successful invasive invertebrates (Kajita & Evans, 2010) and plants (Jelbert et al., 2015).

During the invasion process, invaders may be accompanied by parasites that may originate from (i) its native range, (ii) an intermediate location during the invasion process or finally (iii) the invaded area (Chalkowski et al., 2018). Co-introduction of invasive species in a newly colonized area with their parasites leads to the infection of native species also referred as parasite spillover (McManus et al., 2021). Invasive species may also acquire the parasite from native species leading to parasite dilution across a wider range of hosts. In contrast, they may facilitate the circulation of native parasites between native species and contribute to parasite spillback. For instance, trematode parasite spillover and spillback could have been responsible for hippopotamus parasite spread in Zimbabwean artificial lakes (Schols et al., 2021). Indeed, liver fluke caused by *Fasciola nyanzae* parasites and transmitted to hippotamus by the native snail *Radix natalensis* have also been vectorized afterwards by two invasive snail species (*Radix plicatula* from Asia and *Pseudosuccinea columella* from North America). Meanwhile, a parasite amphistome (not yet reported in Africa) responsible for stomach fluke could also have been imported to artificial lakes by the Asian vector *R. plicatula*. Following introduction, invasive species may escape the parasite colonizing them in their native or intermediate areas. This last process has been reported for bdelloid rotifers (C. G. Wilson & Sherman, 2013). Those microscopic invertebrated escape their fungal parasite when they colonized new habitat patches with higher exposition to drought and wind. All those exemplified events contribute to exacerbate the performance of recently introduced species and increase the probability of invasion success by releasing parasite pressure on its host.

The Asian tiger mosquito *Aedes albopictus* is competent to transmit at least 19 viruses such as Chikungunya, Dengue or Zika (Lequime et al., 2020; Paupy et al., 2009), and filarial nematodes (Cancrini et al., 2003) to humans and animals. Originating from South and East Asia, this vector has been increasing rapidly its distribution all over the world since 1980's (Goubert et al., 2016) and half of the human population could be exposed to it in a near future (Kraemer et al., 2019). Intermediate and long distance mosquito dispersal is strongly influenced by human-aided egg and adult transportation of mosquitoes (*e.g.* transportation of adults in car cabin, freight transport of tires or lucky bamboo colonized by desiccated mosquito eggs) (Bennett et al., 2019; Eritja et al., 2017; Madon et al., 2002; Medley et al., 2015; Miller & Loaiza, 2015; Schmidt et al., 2017; Vega-Rúa et al., 2020). However, local dispersal (~0.75-1km per generation) of mosquitoes can also be mediated through adult flight (Vavassori et al., 2019). Several studies have suggested that genetic drifts or population size reductions due to founder effects have occurred after local introductions of *Ae. albopictus,* but the population size increases dramatically only few years after (Kambhampati et al., 1991; Minard et al., 2015; Sherpa et al., 2020; Gojković et al., 2019; Schmidt et al., 2020).

Natural *Ae. albopictus* populations are highly infected by an Apicomplexan gregarine parasites belonging to the *Ascogregarina* genus (Thongsripong et al., 2018). Various species of *Ascogregarina* are colonizing mosquitoes with a strong host specificity (Lantova & Volf, 2014). The species *Ascogregarina taiwanensis* is specifically associated with *Ae. albopictus* even though experimental or natural spillover of this parasite to unnatural mosquito host species were reported (Erthal et al., 2012; Garcia et al., 1994; Levine, 1988; Munstermann & Wesson, 1990; Reeves & McCullough, 2002). The biological cycle of this parasite is symmetric with the mosquito development (Tseng, 2007). Oocysts are ingested by first instar larvae in water containers and replicate into larvae then pupae until adults release them. Those parasites can be horizontally transmitted either when adults die, defecate or emerge within larval habitats. *As. taiwanensis* may have strong ecological consequences for its mosquito host although it is considered as a weak parasite. Indeed, the presence of the parasite was shown to have detrimental effects on mosquito traits such as development at high parasite density or survivorship when food is limited (Aliabadi & Juliano, 2002a; Comiskey et al., 1999a; Soghigian & Livdahl, 2017). Moreover, studies reported either a decrease in the interspecific competitiveness of mosquitoes or an increase in their aggressiveness toward competitors (Soghigian et al., 2017; Tseng, 2007). However, released *Ascogregarina* oocysts can also infect and kill unnatural hosts while they poorly kill their own host (Garcia et al., 1994; Tseng, 2007). As an example, it induces 76.1% mortality in *Ae. taeniorhynchus*.

In this study, we show evidences of *As. taiwanensis* parasite clearing following the introduction of the Asian tiger mosquito in France with a probable shift in parasite genotypes throughout the worldwide invasion process. To explain the parasite dynamics, we first hypothesized that the decrease in population densities following the mosquito introduction in new areas might affect the parasite inter-individual transmission. Despite a generally greater transmission probability of parasites at high densities, some species such as in the African armyworm *Spodoptera exempta* evolved toward density-dependent prophylaxis (DDP) that leads to an induction of the immune system limiting the parasite multiplication in individuals reared at high densities (K. Wilson & Cotter, 2008). The low host density in founder population should reduce DPP thus promoting parasitism, we then hypothesized that *Ae. albopictus* DDP toward *As. taiwanensis* should be limited. Finally, we hypothesized that new populations are mainly founded by uninfected mosquitoes. This could occur though active local dispersal of the mosquitoes if the flight activity is altered by the parasite or if passive long-distance dispersal through human transportation affects the parasite lifecycle. To test those non-exclusive hypotheses, we asked three questions (i) Does the host density decrease following population introduction affect the parasite dissemination? (ii) Does the parasite limit the mosquito local active dissemination through flight? (iii) Does long distance

dissemination through human-aided egg transportation limit the parasite maintenance? Combining our results with previously published data, we suggest that both populations density decrease following founding events and human-aided transportation contribute to this observed parasitism escape. Furthermore, our results suggest that the parasite recolonizes the mosquito with high densities two years after introduction and that it may improve mosquito local dispersal afterward.

Material and methods

Sampling collection

DNA mosquito extracts from adult males and females from 7 countries and 17 sites (see **Table S1**). Live mosquito samples from different locations across the world were collected with aspirators, net and BG-traps. They were identified using morphological characteristics (Rueda, 2004), stored in 70% ethanol during transportation and frozen until use. Information related to site, country, year of collection, sex, climate, GPS coordinates and the population age (*i.e.* time between population introduction and sample collections) are reported in Table S1. This last information was obtained from local entomological surveillance services in France (EID and EIRAD), as well as from published reports in Italy (Knudsen et al., 1996), Spain (Collantes et al., 2015) and USA (Womack et al., 1995). The date of introduction in Madagascar is relatively uncertain and was estimated to be older than 1904 (Edwards, 1920). Populations from Thailand and Vietnam were considered as natives.

DNA extraction

Mosquito samples were rinsed three time with sterile water, surface disinfected 5min in ethanol and washed 5 times in sterile water. DNA was extracted from each mosquito individually crushed with three 3mm borosilicate beads and under agitation during 60s at 6m/s with a Fastprep homogenizer (MP Biomedicals). Homogenate was then resuspended into a CTAB extraction buffer (2% hexadecyltrimethyl ammonium bromide, 1.4 M NaCl, 0.02 M EDTA, 0.1 M Tris pH 8.0, 0.2% 2-β-mercaptoethanol) and heated for 2h at 60°C. After incubation, 2mg/ml of RNase was added to the extraction buffer and incubated at 37°C for 5 min. DNA was separated from proteins and lipids with an equivalent volume of phenol-chloroform-isoamyl alcohol (25:24:1, v:v:v), vortexed for 15min and centrifuged for 30min at 16,100g. Aqueous phase was harvested and mixed with an equivalent volume of chloroform:isoamyl alcohol (24:1, v:v). The mixture was vortexed for 15min and centrifuged for 30min. The newly separated aqueous phase containing the nucleic acids was harvested. DNA was precipitated with isopropyl alcohol and centrifuged for 30min at 16,100g. DNA pellet were washed with 70% cold ethanol and centrifuged for 30min at 16,100g. Ethanol was

removed and DNA was suspended in 20µl of sterile water. DNA extracts were quantified with a spectrophotometer (SAFAS) and diluted at 15ng/µl.

Metabarcoding of eukaryotic and prokaryotic microbial communities

Prokaryotic and eukaryotic communities were characterized through high throughput sequencing of hypervariable regions from the 16S rDNA and 18S rDNA genes respectively. The V5-V6 region of 16S rDNA gene was amplified using the primers 784F (5'-AGG ATT AGA TAC CCT GGT A-3') and 1061R (5'-CRR CAC GAG CTG ACG AC-3'). The V1-V2 region of the 18S rDNA was amplified using the primers Euk82F (5′ GAA ACT GCG AAT GGC TC 3') and Euk516R (5′ ACC AGA CTT GCC CTC C 3'). Both PCR amplifications were conducted under sterile conditions with 1X of Hotstart 5X Bioamp Master Mix (Biofidal), 0.2µM of each primers, 0.2mg/ml of Bovine Serum Albumin (New England Biolabs), 0.4X of GC rich Enhancer (Biofidal). For 16S rDNA, PCR program started with 10min of initial denaturation at 96°C followed by 35 cycles including 20s of denaturation at 96°C, 1min of annealing at 54°C, 30s of extension at 72°C and ended with a final extension of 10min at 72°C. For 18S rDNA, PCR program started with 10min of initial denaturation at 96°C followed by 35 cycles including 20s of denaturation at 96°C, 30s of annealing at 54°C, 30s of extension at 72°C and ended with a final extension of 10min at 72°C. A total of three negative controls corresponding to blank extractions and PCR were included for each gene. The PCR products were then sent to Biofidal sequencing company for purification and 2x300bp Miseq sequencing (Illumina). A total of 12,836,921 and 14,248,382 reads were obtained from 16S and 18S rDNA respectively and were demultiplexed using the Mothur pipeline (Schloss, 2013). Reads were trimmed based on size (270-310bp and 400-550bp for the 16S and 18S rDNA, respectively) and sequences were conserved if they contained no ambiguous position and if both strands of the paired-end reads aligned together. Chimeras were removed using CHIMERAVSEARCH. The alignment was performed using Silva v.132 database and reads that did not align were filtered out. However, for sequences that matched gregarine, a supplemental local alignment (BLAST) was performed against the Genbank database due to the lack of reference sequences in Silva. Clustering was performed using Opticlust method allowing a maximum dissimilarity rate of 3%. After removing singletons and subsampling, 5,886 and 5,264 OTUs were retrieved respectively for 16S rDNA and 18S rDNA. Contaminant OTUs were removed if their proportion in samples were not at least 10-fold in comparison with controls. Finally, after quality control, data were homogenized by sampling 1,000 and 500 reads respectively for 16S rDNA and 18S rDNA. Miseq sequences have been deposited on XXX under the project number XXX (ongoing process).

qPCR estimation of the eukaryotic and prokaryotic microbial density

Since variations in OTU abundances may be influenced by variations in absolute densities of microorganisms, qPCR essays were performed on samples for which variations in *As. taiwanensis* abundances were observed. Following the metabarcoding analysis and correlation network analysis, three different groups of *Ae. albopictus* microbiota were observed depending on their infection status with *As. taiwanensis* (see Results section). Prokaryotic and Eukaryotic qPCR essays were performed on 7 males and 7 females from each of those three groups using the same primers than in metabarcoding. Reaction mix contained 1X of SYBR Green iTaq (Bio-Rad), 3µM of each primer and 5ng of DNA. PCR cycles were performed on a CFX96 Touch Real-Time PCR detection system (Bio-Rad) following a 3min initial denaturation at 95 °C followed by 40cycles with 5s of denaturation at 95°C and 1min of hybridization and amplification at 60°C. A final denaturation of the PCR products was conducted by progressively increasing the temperature from 60°C to 90°C at 0.11°C/s. The Ct values were used to quantify the copies number of each gene with a standard range of 10^7 to 10^0 genomic DNA copies of *Escherichia coli* DH5-α for the 16S rDNA and a 10^7 to 10^0 genomic DNA copies of *Saccharomyces cerevisiae* CEN.PK2-2 for the 18SrDNA.

PCR-based estimation of Ascogregarina taiwanensis *prevalence*

Detection of *As. taiwanensis* in DNA from field collected individuals was conducted with diagnostic PCR using primers targeting the ITS1-5.8SrDNA-ITS2 intergenic region with the previously published AU (5'-ACCGCCCGTCCGTTCAATCG-3') and AT (5'-GAGAAGCCGTCGTCAATACAGC-3') primers (Morales et al., 2005). The mix contained 1X of Hoststart Master mix (Dutscher, 0.2ng/ml of BSA (Promega), 0.1µM of each primer, 0.1X of GC-Enhancer (Biofidal) and 30ng of mosquito genomic DNA. Reactions were performed with a 10min initial denaturation at 96°C, followed by 35 cycles of amplification including 20s at 96°C, 1min at 50°C, 2min at 72°C and a final elongation of 10min at 72°C. Amplification signals were identified under UV light on a Geldoc 2000 system (Biorad) after 20min of electrophoresis migration at 100V on a 1.5% agarose gel colored with Ethidium Bromide.

Rearing parasitized and unparasitized mosquito lines

A total of ~100 larvae were collected in 2017 in Villeurbanne (N: 45°46'18990'' E : 4°53'24615'') and Pierre-Bénite (N :45°42'11534'' E : 4°49'28743'') in France. This population was grown into larger populations (~5000 individuals) for 15 generations in a Biosafety level 2 insectary at 28°C, with a relative humidity of 80% and a day/night cycle of 18h/6h. Once the eggs were laid, they were immediately placed into water and ~100 adults individuals were crushed and spread to make sure that the parasite was loaded. The water

was never changed until the individuals molt into the 2nd instar since individuals get infected during the 1st instar. This protocol pursued for each generation. Uninfected individuals originate from the same initial population. To ensure that the parasite did not infect them, several actions were performed to shunt the infection path. First, the eggs were not immerged immediately. Then, no contact was permitted between infected individuals and the water in which larvae were reared. Finally, the water was changed during the first instar to reduce the transmission probability. The absence of infection in the uninfected population and presence of infection in the parasitized population was regularly checked by crushing adult individuals in water and observing the mixture at a magnification of x4000 on an optical microscope (Leica).

Testing the influence of mosquito densities on inter-individual transmission

Water containers were maintained with 50ml of sterile water in cages containing either 100 (low density) or 3000 (high density) infected adult individuals during a week to allow them to release oocysts in water. Eggs from an unparasitized population were hatched in sterile water and 50 larvae were added to the container. Larvae were reared at 28°C and fed 1/3 yeast (Biovers) and 2/3 fish food and was provided *ad libitum* to the larvae. Adult males and females were collected at emergence and their parasite density was measured with qPCR from 4 to 10 individuals per sex and replicate. Primers and protocol for *As. taiwanensis* specific qPCR were developed for this study and are detailed in the **supplementary material**. Briefly, reaction mixes for quantification included 0.5µM of AscoJSF (5'-CTMGGCTTGACTTCGGTCT-3') and AscoJSR (5'-TTCCATGCTGGAGTATTCAAGG-3') primers, 1X of iTaq Universal SYBR Green Supermix (Bio-Rad) and 5ng of DNA. The qPCR program included a 5min initial denaturation at 95°C, followed by 40 cycles with 15s at 95°C, 30s at 56°C and 10s at 60°C followed by a progressive denaturation of the qPCR products from 65°C to 97°C at 0.11°C/s. Copy number were estimated based on a standard curve performed with *As. taiwanensis* 18S rDNA cloned in a plasmid. The experiment was reproduced five times.

Testing Density Dependent Prophylaxis (DDP)

To test whether the inter-individual transmission could be related to the density of larvae into the breeding sites, we hatched > 1000 larvae in containers to let them get infected with the parasite released from their parents. We then harvested them with their container water and grew them in new containers at different densities (either 20, 50 or 100 larvae per 100ml of water). Larvae were reared on a shelve providing shadow. When adults emerged, they were collected and their parasite density was measured with qPCR. This experiment was repeated five times and 5 males and females were used for each measurement and

each density. For technical reasons, highest density modality could not be performed for the 5th replicate (*i.e.* poor hatching rate for this replicate). Larval rearing conditions were similar to those in the previous experiment.

Testing the influence of the parasite on active local mosquito dispersal through flight.

Absence of the parasite in recently introduced mosquito populations might be the result of a poor propensity of infected mosquitoes to be active and fly. To test such hypothesis, parasitized and unparasitized adults were collected 2 weeks after their emergence. They were individually stored in a in 25mm Ø activity tube containing a cotton imbibed with a 10% sucrose solution and sealed with cotton. The tube was placed in a LAM25 Locomotor Activity Monitor system (Trikinetics). The system was crossed by 9 infrared beams that register an activity signal whenever the mosquito crosses the beams. Mosquitoes were left for 15min to rest before their activity was measured. The system was placed on a shelve that provides shadow to the mosquitoes since they tend to prefer such habitats in nature (Flacio et al., 2015). Because the activity periodization measured by the LAM system matches the flight activity recorded by video tracking realized on *Ae. albopictus* flight behavior (Wilkinson et al., 2014), and because mosquito walk distance usually do not exceed 20% of total mosquito distance in laboratory experiments (Ziegler et al., 2022), we considered that the recorded activity was mainly due to flight. Two experiments involving 16 parasitized males, 16 parasitized females, 16 unparasitized males and 16 unparasitized females were conducted for 24h in a BSL2 insectary with 18h/6h day/night and 28°C. Activities were binned into a 10min interval. Whenever individuals died before the end of the registration, they were removed from the final dataset.

Testing the ability of the parasite maintenance during passive long-distance transportation on desiccated eggs

Ae. albopictus mosquitoes are often transported as desiccated eggs on containers. Either local or long distance transportation of mosquitoes has previously been showed to occur through transportation of eggs laid on attractive containers (*e.g.* used tires and lucky bamboo) (Bennett, Gómez Martínez, et al., 2019; Cornel & Hunt, 1991; Dalla Pozza & Majori, 1992; Laird et al., 1994; Moore & Mitchell, 1997; Pluskota et al., 2008; Schaffner et al., 2004). Eggs then hatch whenever the containers get flooded after being introduced in a new area.Under optimal conditions, previous studies showed that *Ae. albopictus* eggs can survive a 1 month desiccation period (Juliano et al., 2002). However, we do not know whether such transportation paths could affect the parasite. To estimate that, we let mosquitoes laying eggs on water containers coated with a dark green blotting paper. Since *Ae. albopictus* tends to prefer dark container (Clements, 1999), the blotting paper was preserved in the dark under

optimal conditions (*i.e.* 26°C with a relative humidity of 80%). After respectively 35h, 1 week and 1 month, 100 eggs were harvested from the blotting paper and placed in a water container to hatch. The adults that emerged from those containers were collected. The number of parasites colonizing the adult mosquitoes was estimated with qPCR on 10 males and females for each time point and each replicate. This experiment was repeated five times on five different blotting papers.

Statistical and data analysis

Prevalence data were analyzed with a binomial model and a probit link function (glm, R package stats). Statistical analyses of fixed effects (sex and time from introduction) were performed with a Type II Wald χ^2 test (Anova, R package car). Geographical distance between mosquito populations were calculated based on ellipsoid distance (pointDist, R package enmSdm).

Microbiota diversity has been estimated using taxa number for richness (specnumber, R package Vegan), Shannon index for alpha diversity (diversity, R package Vegan) and Bray-Curtis dissimilarity index for beta diversity (vegdist, R package Vegan). Richness and alpha diversity have been analyzed using generalized linear models (glm, R package lme4) and linear models (lm, R package lme4) respectively, meanwhile beta diversity has been analyzed using permutational multivariate analysis (adonis2, R package vegan). Microbiota composition and abundance have been represented as a heatmap (Heatmap, R package Complexheatmap).

The microbiota abundance correlation networks were performed with a homemade *python* code and visualized with the *pyvis* library. Briefly, the 200 most frequently retrieved OTUs for each community (prokaryotic and eukaryotic) were selected and their paired non-parametric correlations were tested. If the Spearman correlation (Rs) value was >0.25 (positive correlation) or <-0.25 (negative correlation), a linked was added between the two pairs. An automatic positioning algorithm was used based on mass-spring models. In this model the links plays a spring role which tend to bring the OTUs together while the OTUs play a role of opposite mass charges that are constantly repelled from each other. The algorithm used iterations to minimize the energy of the system.

The phylogenetic analysis of *As. taiwanensis* OTUs (based on an alignment of the most abundant sequences in each OTU) was performed with Seaview and the Muscle algorithm using other members of the Apicomplexa phylum. Phylogenetic trees were then generated with a Maximum Likelihood method, an HKY evolutionary model and 100 bootstraps (performed with Seaview and represented with Figtree).

For the founder effect and desiccation experiments, the densities of *As. taiwanensis* per mosquito were modeled with a linear mixed model and a negative binomial generalized

mixed model respectively (lmer and glmer.nb, R package lme4). The experiment replicates were used as random factors. The fixed effects (sex, density or desiccation time) and their interaction were tested with type II Wald χ^2 test (Anova, R package car). For the Experiment 2, flight activities over the entire recording period were represented as a heatmap (Heatmap, R package Complexheatmap). The total activities for each mosquito were modeled with a negative binomial generalized linear mixed model since data were zero inflated (glmer.nb, R package lme4). Experiment replicates were used as a random variable. Fixed effects (sex and parasite status) and their interaction were tested with type II Wald χ^2 test (Anova, R package car).

Results

Recently introduced mosquitoes harbor lower prevalence of As. taiwanensis

The parasite prevalence was estimated on 598 adult female and male mosquitoes sampled across 17 populations by diagnostic PCR (**Table S1**; **Fig. S1**). The impact of different fixed effects on *As. taiwanensis* prevalence has been investigated and the associated statistical analyses have been summarized in **Table 1**. Significant interaction was evidenced between the sex of mosquitoes and their localization when combined together in a single model. Because of this significant interaction, the two sexes were then analyzed separately. Both males and females showed significant parasite prevalence variations across sampling sites. To explain this pattern, we further tested the influence of the site climate, geographical distance and population age on the parasite prevalence. No correlation was found between parasite prevalence variation and geographical distance or climate between mosquito populations (**Table 1, Figure S2**). However, it was positively correlated with the mosquito population age (**Figure 1**).In recent mosquito populations (< 2 years), the prevalence of *As. taiwanensis* ranged from 0 to 25%, while it ranged from 47% to 100% in populations that were introduced for more than 6 years. This effect was specifically strong on French sites (that are geographically closed but harbor high difference in parasite load correlating with differences in population ages).

Ascogregarina taiwanensis *abundances correlate with the microbiota composition, the mosquito origin and the population age*

To compare dynamics of *As. taiwanensis* abundances within the microbiota among different mosquito populations, a metabarcoding analysis was conducted on a subset of 248 male and female mosquitoes (**Table S1**). The eukaryotic and prokaryotic microbiota of *Ae.*

albopictus was dominated by two OTUs classified as *Wolbachia* (**Figure 2A**) and three OTUs classified as *Ascogregarina* (**Figure 2B**). The three *Ascogregarina* OTUs could be confidently classified within the *Ascogregarina taiwanensis* species based on a maximum likelihood phylogeny conducted on the same 18S rDNA region with closely related taxa (**Figure 3A**). Interestingly, 9 different OTUs of *Ascogregarina* were identified among the complete dataset meanwhile only 2 OTUs were identified for *Wolbachia*. *Ascogregarina* showed a prevalence of 77.4% and an average abundance of 29.2% among the eukaryotic microbiota while *Wolbachia* showed a prevalence of 93.1% and an average abundance of 39.4% among the prokaryotic microbiota. Other OTUs identified as *Aureobasidium* and *Penicillium* genera for eukaryotes or as *Cutibacterium*, *Staphiloccocus* and *Acinetobar* genera for prokaryotes were also prevalent and could be considered as part of the core microbiota of *Ae. albopictus*. Study of factors involved in variation of microbiota composition (*i.e.* β-diversity) between individuals revealed that origin of the mosquito was the most important structuring factor in interaction with the sex at a lower magnitude (see **Table S2**). In other terms, the microbiota of mosquitoes strongly differed among localization and slightly independently for each sex. Both eukaryotic and prokaryotic microbiota dissimilarities were also correlated with the age of the population (**Figure S3**), climate (**Figure S4**) and geographical distance between individuals (**Figure S5**) for each sex.

Ascogregarina taiwanensis *most abundant gentoypes define microbiota subgroups in networks and are correlated with mosquito population years after introduction*

The three most abundant *Ascogregarina taiwanensis* OTUs were part of the 8 OTUs that correlated the most with the introduction of the mosquito but Otu000001 variations were symmetrically opposite from Otu000002 and Otu000003 in the dbRDA axis (**Figure 3B**). This pattern was confirmed with correlations networks that showed two independent networks of positive correlations between OTUs involving *Ascogregarina* OTUs (**Figure 3C**). The first network involved the dominant *Ascogregarina* (Otu000001) and fifth other underrepresented *Ascogregarina* OTUs (Otu000070, Otu000071, Otu000085, Otu000092, Otu000096) while the second one involved solely the two others dominant *Ascogregarina* OTUs (Otu000002 and Otu000003). We then modelled the variations of those three OTUs with the time after mosquito introduction, sex and their interaction. Through likelihood ratio test, we showed that none of those OTUs significantly differed between sex but Otu000002 and Otu000003 showed a significant covariation with the time after mosquito introduction (see Table S2). Otu000002 and Otu000003 increased in abundance in old populations while Otu000001 did not significantly vary with the age of the population (**Figure 4**). Since abundance is defined as the proportion of sequences occupied by a given OTU in its community (eukaryotes in this case), we performed qPCR to estimate the absolute density of microorganisms for each

marker used in this study. DNA quantities were limited so we could not perform this test for all the 248 samples, we separated the dataset in 3 eukaryotic communities based on the metabarcoding (**Figure 2B**). The first group (60 individuals) was dominated by the most prevalent OTUs of *As. taiwanensis* (Otu000001), the second (43 individuals) by two different OTUs of *As. taiwanensis* (Otu000002 and Otu000003), and the last one (84 individuals) by neither of those three OTUs. The eukaryotic and prokaryotic microbiota of 14 individuals (7 males and 7 females) from each group were then analyzed by qPCR. The quantitative analysis (**Figure S6**) showed that microbiota load varied between groups ($F_{2,36}$=3.76 ; p=0.033) and the second group counted less microbes than the third group (t-ratio=-2.34; p=0.05) but no significant differences were evidenced between Groups 1 and 2. Therefore, the dominance of Otu000002 and Otu000003 in native populations and Otu000001 in old but non-native populations is not likely to be due to changes in absolute densities of those dominant OTUs. Interestingly, the individuals that were not colonized by those dominant *As. taiwanensis* OTUs did harbor a dense eukaryotic microbial community compared to populations dominated by Otu000002 and Otu000003.

Founder effects can reduce the amplitude of parasite infections due to low mosquito population densities and an absence of Density-Dependent Prophylaxis (DDP)

Since recently introduced populations are often founded by a small number of individuals, density dependent effects may contribute to a reduction of the parasite transmission. The number of released oocysts in larval water correlated with parasite transmission (Soghigian & Livdahl, 2017). What was unclear was whether a higher density of infected adults transmitting oocysts to larvae would be correlated with a higher parasite transmission. When the number of adults that releasing oocysts in water was increased, we observed a consistent increase in the density of parasites colonizing individuals emerging from the water habitat (**Figure 5A, Table 1**). We also noticed that emerging females harbored more parasite oocysts than males. In order to test whether differences in larval density would modify the parasite multiplication, we separated 1rst instar infected larvae into three batches of 20, 50 and 100 larvae per 100ml and repeated the experiments five times (**Figure 5B, Table 1**). The parasite success was not significantly modified by larval densities either in males or in females (**Figure 5B, Table 1**).

The parasite is not likely to impair mosquito dispersion through flight since parasitized individuals are more active.

The flight behavior of uninfected and infected mosquitoes was assessed using a Locomotor Activity Monitor. We recorded individually the number of movements of parasitized and unparasitized mosquitoes during 24h (**Figure S7**). Contrarily to our expectations, parasitized mosquitoes were significantly more active than unparasitized ones. Parasitized mosquitoes moved on average 73.8 times per day meanwhile unparasitized conspecifics moved only 15.5 times per day (**Figure 6, Table 1**). Therefore, those results suggest that the low infection rate of mosquitoes in young populations is not related to a higher propensity of uninfected individuals to disperse by flight. On the contrary, those experiments show that parasites could empower mosquito invasive capacities at the local scale due to a higher fly activity of infected individuals.

Parasitic success decreases over time when eggs are desiccated during human-aided transportation

To evaluate whether egg desiccation may restrain the parasite infectivity through long distance transportation, we collected eggs from five containers and splited them into three groups of 100 eggs that we respectively flooded after 1 day, 1 week and 1 month of desiccation. The parasite infection was finally assessed after adult's emergence by qPCR on 10 males and 10 females per container and per condition. For both males and females, the parasite load in adults significantly decreased over time after desiccation (**Figure 7, Table 1**). Therefore, the harsh conditions occurring along travelling process of the mosquito eggs are probably involved in the fall of *As. taiwanensis* prevalence in short-aged populations.

Discussion

The dominant parasite Ascogregarina taiwanensis *is positively correlated with the population age*

In this study, we have evidenced that *As. taiwanensis* is a dominant member of the mosquito microbiota in terms of prevalence and density similarly to the parasitic bacteria *Wolbachia*. This was previously suggested for local populations from Thailand (Thongsripong et al., 2018), but the eukaryotic microbiota has largely been understudied in this mosquito species like in many other models (Zha et al., 2022).

Interestingly, we showed that three dominant *As. taiwanensis* OTUs occur among mosquito populations. One OTU (Otu000001) does not show consistent variations in abundance according to population age while the two others (Otu000002 and Otu000003) are more abundant in old populations. Since the ecology of those OTUs and the nature of their

interaction with the host is unknown, we can only speculate that potential genotype x genotype x environment interactions do influence the dynamics of *As. taiwanensis* and its host in natural habitats. Genotype x genotype interactions have previously been suggested for this model with a reported increase of parasite performance in its sympatric host compared to allopatric ones (Tseng, 2017). Other apicomplexan have been shown to coevolve with their hosts such as *Plasmodium spp*. with humans (Evans & Wellems, 2002) or *Eimeria spp.* with rabbits (Kvicerová et al., 2008) and marine invertebrates (Xavier et al., 2018). Dynamic of those different *As. taiwanensis* molecular species could also be correlated with the host population dynamics. Several studies have reported a high variation in genetic diversity and differentiation amongst *Ae. albopictus* populations (Duong et al., 2021; Latreille et al., 2019). Genetic dynamics of *Ae. albopictus* populations have been driven by multiple introductions, genetic drifts and founder effects during their invasion process (Gao et al., 2021; Hall et al., 2022; Konorov et al., 2021; Latreille et al., 2019). The weak gregarine diversity (limited density of gregarines from Otu000002 and Otu000003) in recently introduced populations could then be related to those events since the parasite cannot reproduce outside of its host. In addition, some gregarine genotypes might not be well adapted to stresses encountered during transportation or new environmental conditions experienced in the invaded areas. Further studies are still needed to decipher the intricate relationships between genotypes of *As. taiwanensis* and its host.

Regardless of the genotype, we reported a positive correlation between *As. taiwanensis* infection level and *Ae. albopictus* population age with recently introduced mosquito populations harboring the lowest parasite prevalence and load. Even though the populations that showed the most important variations in prevalence were in France, we speculate that this pattern might be more global for several reasons. First, none of the older populations sampled in distant parts of the world harbored low prevalence as observed in recently introduced ones from France. Secondly, the geographical distance and climate did not correlate with the parasite prevalence since geographically close populations with different age of introduction showed strong divergences in parasite prevalence. Finally, this observation is consistent with parasitism escape reported in American *Ae. albopictus* populations more than 20 years ago after introduction events (Aliabadi & Juliano, 2002b; Blackmore et al., 1995). A first study conducted on 27 populations from Florida showed that 33% of sites in which the mosquito was introduced for less than 3 years were infested by the parasite while 83% of those colonized by older mosquito populations were infested (Blackmore et al., 1995). Upper north in the USA, the parasite prevalence and load were lower in recent populations (<2 years) from Peoria (USA) compared to more established ones (>14 years) from Saint-Louis (USA). Under controlled conditions, they did not find any impact of parasitism on *Ae. albopictus* survivorship although unparasitized females

developed faster and unparasitized larvae were more efficient to compete with *Ae. triseriatus*, an endemic mosquito species.

Mosquito population shift in densities during the invasion process likely drives the parasite dynamics

In other host-parasite systems such as snail-trematode, the host density has been shown to be a good predictor of parasite colonization success (Buck et al., 2017). Such phenomena are often predicted to occur for horizontally transmitted parasites since inter-individual contacts leading to higher transmission probability increases with population density (Watve & Sukumar, 1995). Since inter-individual horizontal transmission of parasites is mediated through oocyst released from adults in water habitats, one can expect that a reduced number of mosquitoes invading a new area would result in a limited number of parasites released in the breeding sites and colonizing the adults. Transmission is also likely to be facilitated by the gregariousness of mosquito larvae. Previous study on *As. taiwanensis* evidenced that the parasite oocyst load released in water conditions the degree of host infection (Soghigian & Livdahl, 2017). In this study, we have complemented those results by showing that the number of adult mosquitoes releasing parasites positively increases the number of parasites efficiently colonizing unparasitized conspecifics emerging from larval habitats. Interestingly an interseasonal mosquito and *As. taiwanensis* dynamics have been shown to be correlated over the year (Garcia et al., 1994). Those results converge toward the importance of high mosquito population densities for an efficient parasite transmission. Independently from the probability of parasite acquisition, previous studies on gregarious insects showed that an adaptive response to host high density leads to a reduction of the parasite susceptibility through the enhancement of their immune system (K. Wilson & Cotter, 2008). This phenomenon is also named Density-Dependent Prophylaxis (DDP). For instance, in response to density raise, the bumble bee *Bombus terrestris* (Hymenoptera) and the velvet bean caterpillar *Anticarsia gemmatalis*, increase their phenoloxydase activity (immune response leading to a melanization process) (Ruiz-González et al., 2009; F. W. S. Silva et al., 2016). In this study, we have shown an absence of larval density-mediated modulation of the parasite infection cycle. Although the immune response has not been tested, this suggests an absence of DDP in *Ae. albopictus* larvae toward *As. taiwanensis*. We can then predict that the parasite transmission drastically increases with the population size without being slowed down by DDP.

The parasite is not likely to alter the mosquito local dispersal but rather increase it

We have highlighted the impact of the different invasion processes in the host-parasite relationship. Contrarily to our hypothesis, local mosquito dispersal through flight has

been shown to be improved by parasitism. Local flight distance of field populations is generally limited to a few hundred meters (Vavassori et al., 2019) and remains a secondary factor to explain the tiger mosquito invasiveness. This hypothesis of gregarine dispersal through local flight is actually highly debatable due to opposite examples such as the monarch butterfly. Indeed, the infectivity of the gregarine *Ophryocystis elektroscirrha* to monarch butterfly *Danaus plexippus* has been shown to reduce during winter migration (Dargent et al., 2021). In this model, the gregarine infection has been proved to reduce host wing mass and wing resistance (Davis & Roode, 2018). In other models such as the dragonfly *Libellula pulchella*, gregarines are also negatively affecting the flight performance by decreasing the concentration of muscle myosin and impairing muscle contractile performance (Marden & Cobb, 2004; Schilder & Marden, 2007). The results reported here are preliminary. Indeed, laboratory experiments on mosquitoes behavior using locomotor activity monitor do not take into account various stimuli (e.g. breeding sites, hosts…) from the environment that might differentially enhance the local dispersal of mosquitoes (Girard et al., 2021; Wooding et al., 2020). Other factors such as the mosquito age and nutrition would also be considered in the future while previous studies showed that the activity varies according to those factors (Kaufmann et al., 2013). If our result is confirmed under more complex conditions, this can suggests that mosquitoes that have been colonized by the parasite after introduction might be more prompt to locally disperse.

Conditions during human-aided long-distance dispersal might favor parasitism escape

The Asian tiger mosquito invasive process is mainly induced by human-mediated large scale dispersal as previously discussed (Roche et al., 2015) and is facilitated by egg ability to enter diapause and resist to desiccation (Bonizzoni et al., 2013). We demonstrated the negative impact of desiccation on parasite maintenance. Similar results were obtained on gregarine *Apolocystis elongata* which harbors a reduced infectivity toward its earthworm host *Eisenia foetida* three weeks after being released on a dry soil (Miles, 1962). Since transportation of desiccated eggs is a major path driving the mosquito introduction (Bennett, Gómez-Martínez, et al., 2019; Eritja et al., 2017; Madon et al., 2002; Medley et al., 2015; Miller & Loaiza, 2015; Schmidt et al., 2017; Vega-Rúa et al., 2020), human-aided transportation could be a consistent factor explaining *Ae. albopictus* parasitism escape after mosquito population introduction. However, it is not likely to be the only factor causing this phenomenon since intermediate distance mosquito dispersal has also been shown to be mediated through adult mosquitocar cabin transportation, a path that can hardly be related to parasitism escape (Eritja et al., 2017). Therefore, we suggest that the parasite decrease after introduction is mainly driven by mosquito demographic variations but that human-aided transportation can exemplify this phenomenon.

Ecological factors explaining the dynamic of parasitism still need to be investigated

Recently, a mathematical model predicted the Asian tiger mosquito competitiveness against the tree hole mosquito *Ae. triseriatus* in presence of gregarine parasites (Stump et al., 2021). In most of scenarii, *Ae. albopictus* dominates *Ae. triseriatus* but parasitism decreases its dominance due to a longer development time, in accordance with other studies (Comiskey et al., 1999b). The authors concluded that parasitism reduces *Ae. albopictus* competitiveness. Selective pressure induced by competition might explain the loss of parasites in recently introduced *Ae. albopictus* populations since its presence might restrain the population settlement probability. Another hypothesis to explain the parasitism escape is a dilution effect when *Ae. albopictus* colonizes new areas. Both parasite prevalence and abundance could be reduced due to a wider range of potential host (induced by native mosquito species). Indeed, they can colonize first instar larvae of other mosquito species but rarely achieve to complete their life cycle and replicate inside them (Lantova & Volf, 2014). To the best of our knowledge, there is no study focusing on a potential dilution of *As. taiwanensis* out of *Ae. albopictus*. However, this was suggested by field surveys reporting that the prevalence of *As. taiwanensis* colonizing *Ae. albopictus* was higher when the mosquitoes did not share breeding sites with *Ae. aegypti* (Dos Passos & Tadei, 2008). *Ae albopictus* is often reported as a strong competitor toward other mosquitoes (Bagny Beilhe et al., 2013; Costanzo et al., 2005; de Oliveira et al., 2017; Juliano, 1998; Kesavaraju et al., 2014; O'Donnell & Armbruster, 2007). Therefore, the parasite prevalence dynamics in young populations could be due to an evolution of the dilution effect resulting from a higher diversity of mosquitoes in breeding sites at the early stage of the colonization process that decrease over the time due to interspecific competition. Some gregarines have developed strategies to minimize the impact of parasite dilution. As an example, the infectivity of *As. barreti* toward its natural host *Ae. triseriatus* is higher when another mosquito species is present in the breeding site (McIntire et al., 2021 b). Field studies on the arthropod's community sharing breeding sites with recent and ancient populations may help to find relevant alternative hosts to test this hypothesis.

Conclusion

In this study, we provide a new insight into the *Ae. albopictus* ecology of host-gregarine relationship by combining field observations and laboratory experiments. The parasite is very abundant and prevalent in installed mosquito populations meanwhile it is poorly associated to recently introduced populations. The mosquito invasion process is likely a major reason explaining these dynamics due to founder effects and desiccation occurring

during humain-aided transportation of mosquito eggs. These results illustrate an example of human-aided parasitism escape. In addition, the increased mosquito flight activity in parasitized individuals could be paradoxically favor the mosquito local dispersal when the parasite recolonizes invasive populations. This study, therefore illustrates the importance of studying interacting metacommunities to improve our knowledge of biological invasion consequences. Many questions remain unanswered concerning the molecular mechanisms involved in *As. taiwanensis* and *Ae. albopictus* interactions. Deciphering parasite-mosquito molecular crosstalk through transcriptomic and metabolomics analysis could help us to better understand the mechanisms underlying its impact on mosquito fitness and flight activity.

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Table 1. Fixed factor impact on *Ascogregarina taiwanensis* prevalence, abundance and load associated with the Asian tiger mosquito natural populations and laboratory

Response Variable	Fixed Effect	Experiments	χ^2 stat	Subsampled datasets	*p*-value	Spearman correlation index
Prevalence (GLM)	Origin		280.664		<0.0001***	
	Sex		0.462		0.496585	
	Origin x Sex		26.046		0.008976**	
	Origin		198.09	Female	<0.0001***	
	Origin		107.61	Male	<0.0001***	
Prevalence (GLM)	Climate		1.0230e-12		1	
	Climate x Sex		-4.8772e-11		1	
Prevalence (Spearman correlation)	Age of the population			Female	<0.0001***	0.5022401
				Male	<0.0001***	0.3645566
Δ Prevalence (Mantel correlation)	Geographical distance			Female	0.2026	0.05730781
				Male	0.2027	0.07085295
Ascogregarina/Otu000001 abundance (GLM)	Years after introduction		0.6940		0.4409	
	Sex		0.3500		0.5541	
	Years after introduction x Sex		2.0737		0.1499	
Ascogregarina/Otu000002 abundance (GLM)	Years after introduction		19.1842		1.187e-05***	
	Sex		1.3936		0.2378	
	Years after introduction x Sex		0.4375		0.5083	
Ascogregarina/Otu000003 abundance (GLM)	Years after introduction		8.7003		0.003182**	
	Sex		0.0160		0.899290	
	Years after introduction x Sex		2.0092		0.156380	
Parasite load (GLM)	Adult mosquito density	Inter-individual transmission	35.85		<0.0001***	
	Sex		9.03		2.66e-3**	
	Larval mosquito density	Density Dependent Prophylaxis	0.16		0.69	
	Sex	Desiccation resistance	4.4967		0.03416*	
	Egg desiccation		26.32	Female	<0.0001***	
	Egg desiccation		68.81	Male	<0.0001***	
Flight activity (GLM)	Infection status	Flight activity	11.5808		0.0007***	
	Sex		0.4776		0.4894984	

experiments associated

Table S1. *Aedes albopictus* population used in the current study

Country	Origin site	Months of sample	Years of sample	Years after introduction	Sex	n. samples prevalence	n. samples metabarcoding	Climate	GPS coordinates
France	Saint-Priest	July	2012	0	males	8	10	Semi-continental	N : 45°41'49"
					females	28	10		E : 4°53'50'
	Saint-Priest	August	2018	1	males	24	-	Semi-continental	N: 45°43'8'
					females	28	-		E : 4°55'9"
	Oullins	July	2018	1	males	20	10	Semi-continental	N 45°42'42"
					females	18	10		E : 4°48'2"
	Feyzin	July	2018	1	males	18	10	Semi-continental	N: 45°40'25'
					females	22	10		E : 4°51'33"
	Villeurbanne	July	2018	5	males	28	10	Semi-continental	N : 45°46'18"
						30	-		E : 4°53'24"
	Nice	August	2012	7	males	16	-	Mediterranean	N : 43°41'60"
					females	20	10		E : 7°17'33"
	Portes-les-Valence	July	2013	2	males	7	10	Semi-continental	N 44°52'8"
					females	31	10	Mediterranean	E : 4°52'9'
Italy	Turin	September	2011	15	males	17	10	Mediterranean	N: 45°4'13'
					females	13	10		E : 7°41'12"
Spain	Barcelona	July	2013	9	females	22	10	Mediterranean	N: 41°23'9.2'
									E : 2°10'24"
USA	Atlanta	October	2018	33	males	25	10	Humid Subtropical	N : 33°44'56"
					females	24	10		O : 84°23'16"
Madagascar	Diego Suarez	NA	2009	>1005	males	18	-	Humid Tropical < 900m	S : 12°16'51.24"
					females	14	10		E : 49°17'27.092"
	Toamasina	December	2010	>1005	males	22	10	Humid Tropical < 900m	S : 18°8'59.64"
					females	19	10		E : 49°24'8.312'
	Tsimbaza-Park	December	2010	>1005	males	13	10	Tropical > 900m	S : 18°55'40.395"
					females	25	10		E : 47°31'38.5'
Thailand	Chiang Mai	February	2019	Native	males	8	8	Tropical	N 18° 42' 46.1"
									E 98° 56' 17.1"
Vietnam	Hồ-chi-minh	October	2012	Native	females	20	10	Tropical	N : 10°47'19"
									E : 106°42'19"
	Binh Duong	October	2012	Native	females	19	10	Tropical	N : 10°47'19"
									E 106°42'19'
	Vũng Tau	October	2012	Native	males	25	10	Tropical	N : 10°22'26"
									E : 107°4'3"

Table S2. Fixed factor impact on the microbial divervisity associated with the Asian tiger mosquito.

Class	Response Variable	Fixed Effect	Sex	F/χ^2 stat	*p-value*	R^2
Eukaryotic microbiota	Richness (GLM)	Origin		593.99	$<2.2 \times 10^{-16}$ ***	
		Sex		4.01	0.0453*	
		Origin x Sex		181.49	$<2.2 \times 10^{-16}$ ***	
	Alpha diversity (LM)	Origin		7.47	2,738e-13 ***	
		Sex		2.81	0.09495	
		Origin x Sex		1.8457	0.12391	
	Beta diversity (permANOVA)	Origin		7.19	0.001 ***	0.32
		Sex		1.17	0.28	0,00
		Origin x Sex		1.81	0.001 ***	0.04
		Years after introduction	Female	4.7389	0.001 ***	0.22
		Years after introduction	Male	4.8806	0.001 ***	0.24
		Climate	Female	3.8923	0.001 ***	0.08
		Climate	Male	4.7886	0.001 ***	0.13
Bacterial microbiota	Richness (GLM)	Origin		887.33	$<2,2 \times 10^{-16}$ ***	
		Sex		0.12	0.7319	
		Origin x Sex		162.12	$<2,2 \times 10^{-16}$ ***	
	Alpha diversity (LM)	Origin		15.14	$<2,2 \times 10^{-16}$ ***	
		Sex		0.78	0.38	
		Origin x Sex		18.46	0.0087**	
	Beta diversity (permANOVA)	Origin		7.85	0.001 ***	0.32
		Sex		7.08	0.001 ***	0.02
		Origin x Sex		3.57	0.001 ***	0.07
		Years after introduction	Female	3.7418	0.001 ***	0.19
		Years after introduction	Male	4.2892	0.001 ***	0.21
		Climate	Female	5.6248	0.001 ***	0.11
		Climate	Male	8.9578	0.001 ***	0.16

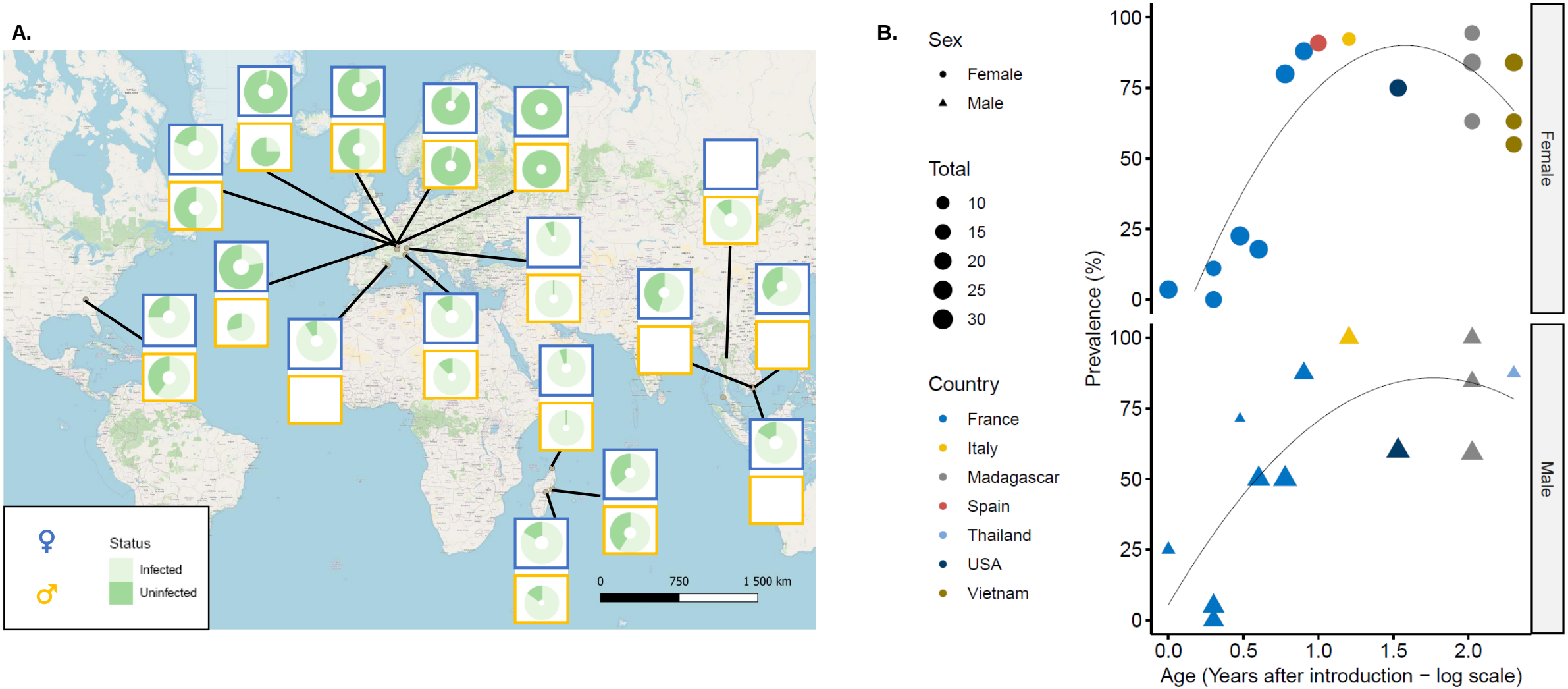

Fig. 1. Parasite prevalence among worldwide mosquito populations. (A) Geographical distribution of infected (dark green) and uninfected (light green) female (blue square) and male (yellow square) mosquitoes. The chart sizes is proportional to the number of individuals tested. An absence of chart corresponds to absence of individuals. (B) Correlation between parasite prevalence (*i.e.* proportion of infected individuals) and the population ages (number of years separating the mosquito introduction from the collection date) for both females (circles) and males (triangles) from different countries (colors). Each dot represent a sampling site. The native populations were arbitrarily set to 200 years to be ordered at last in the graph.

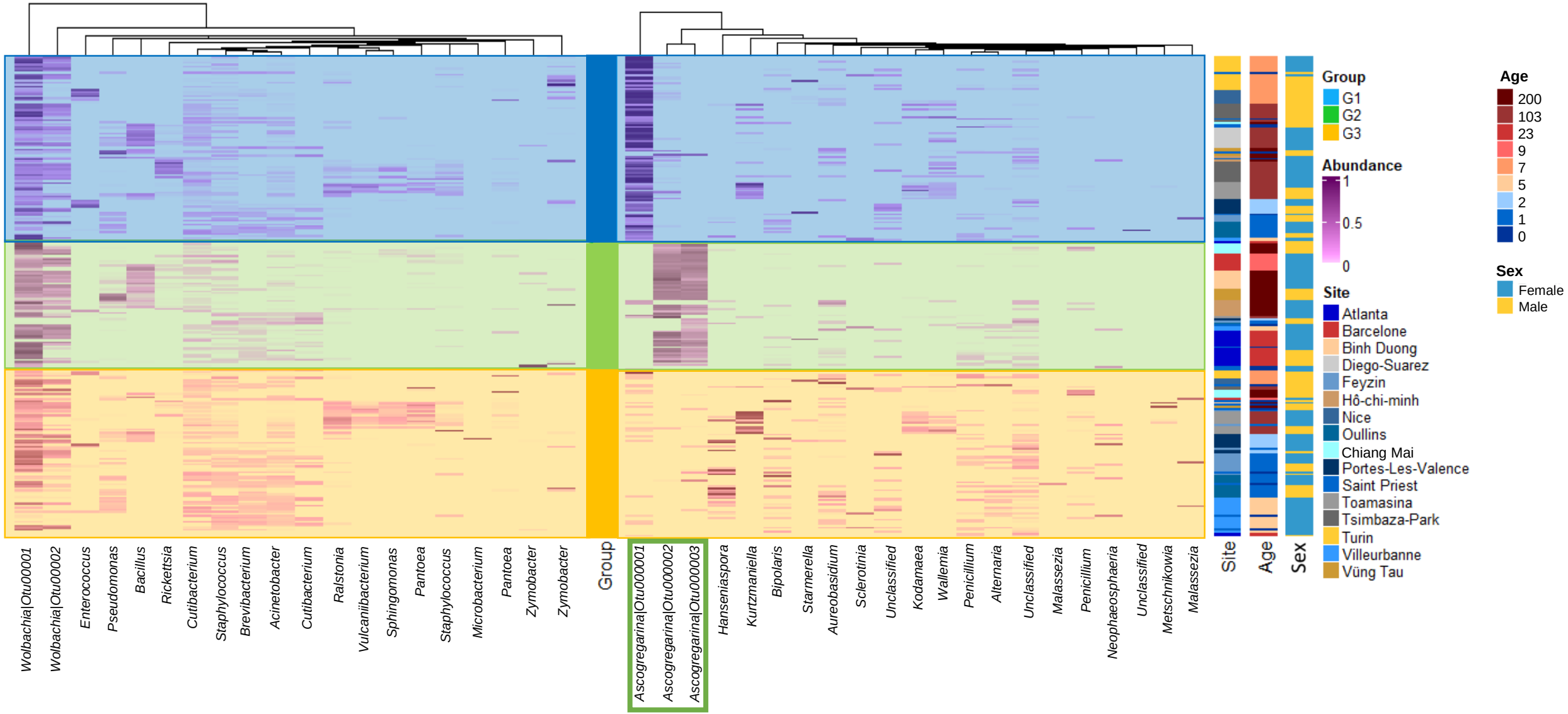

Fig. 2. Heatmap of the microbiota associated with *Ae. albopictus*. The 20 most abundant (A) prokaryotic and (B) eukaryotic OTUs were represented in columns. The number attached to *Wolbachia* and *Ascogregarina* OTUs corresponds to the OTUs number in the following analysis. The colour scale show the OTU abundance (pink). Rows are classified depending on the central scale that represent three different groups based on eukaryotic community dissimilarity (Bray-Curtis distance) and that correlates with *As. taiwanensis* taxa domination. Individuals belonging to the G1 group are dominated by *Ascogregarina* Otu000001, those belonging to the G2 group are dominated by *Ascogregarina* Otu000002 and *Ascogregarina* Otu000003 and those belonging to the G3 group are not dominated by *Ascograrina* OTUs. Three additional scales show colors corresponding to the individuals' origin site, age (years after introduction), and sex.

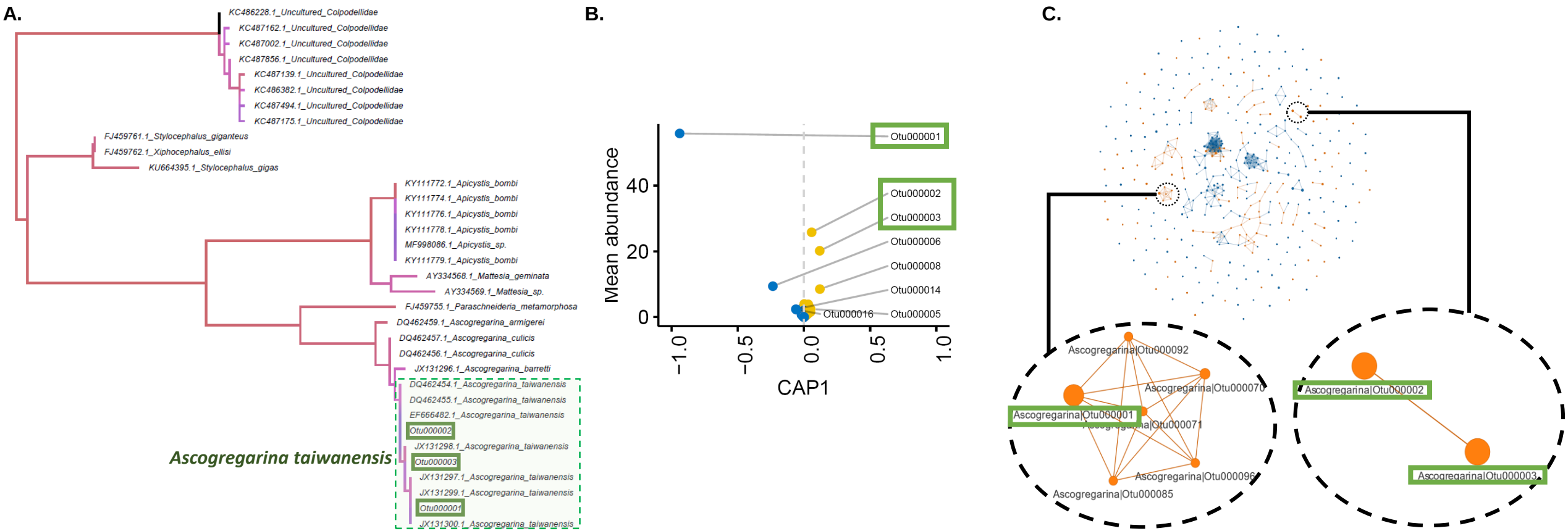

Fig. 3. Three dominant genotypes of *As. taiwanensis* are independently covarying with the population and the time spent after population introduction. (A) Phylogenetic analysis revealed the belonging of OTU identified as Gregarinasina to the species *Ascogregarina taiwanensis*. (B) Mean abundance of eukaryotic OTUs according to the coordinate of those same OTU on a canonical analysis of principal coordinates (CAPSCALE) highlighting correlations between OTUs and the age of the population. (C) A network analysis (R>0.25) showing positive correlations between microbial OTUs (eukaryotes are represented in yellow while prokaryotes are in blue and each link represents a correlation with ρ>0.25). The left network in the zoom is dominated by *Ascogregarina* Otu000001 and is correlated with minor *Ascogregarina* OTUs. The right network in the zoom shows correlations between *Ascogregarina* Otu000002 and Otu000003.

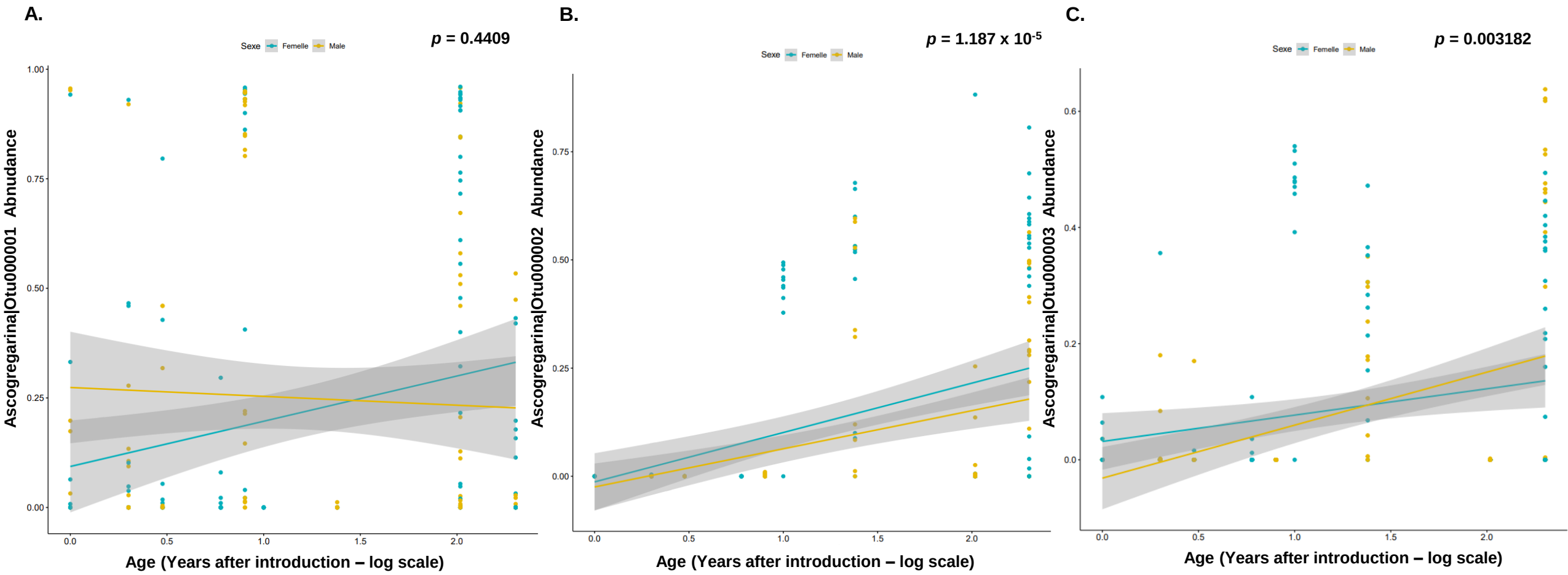

Fig. 4. Correlations between *Ascogregarina* OTUs abundance and the mosquito population age. For female (in blue) and male (in yellow) mosquitoes, the age of the mosquito populations and the abundance of the three dominant *Ascogregarina* (A) Otu000001, (B) Otu000002 and (C) Otu000003. The p-value represent the significance of the explanatory variable (years after introduction). The native population was arbitrarily set to 200 years after introduction.

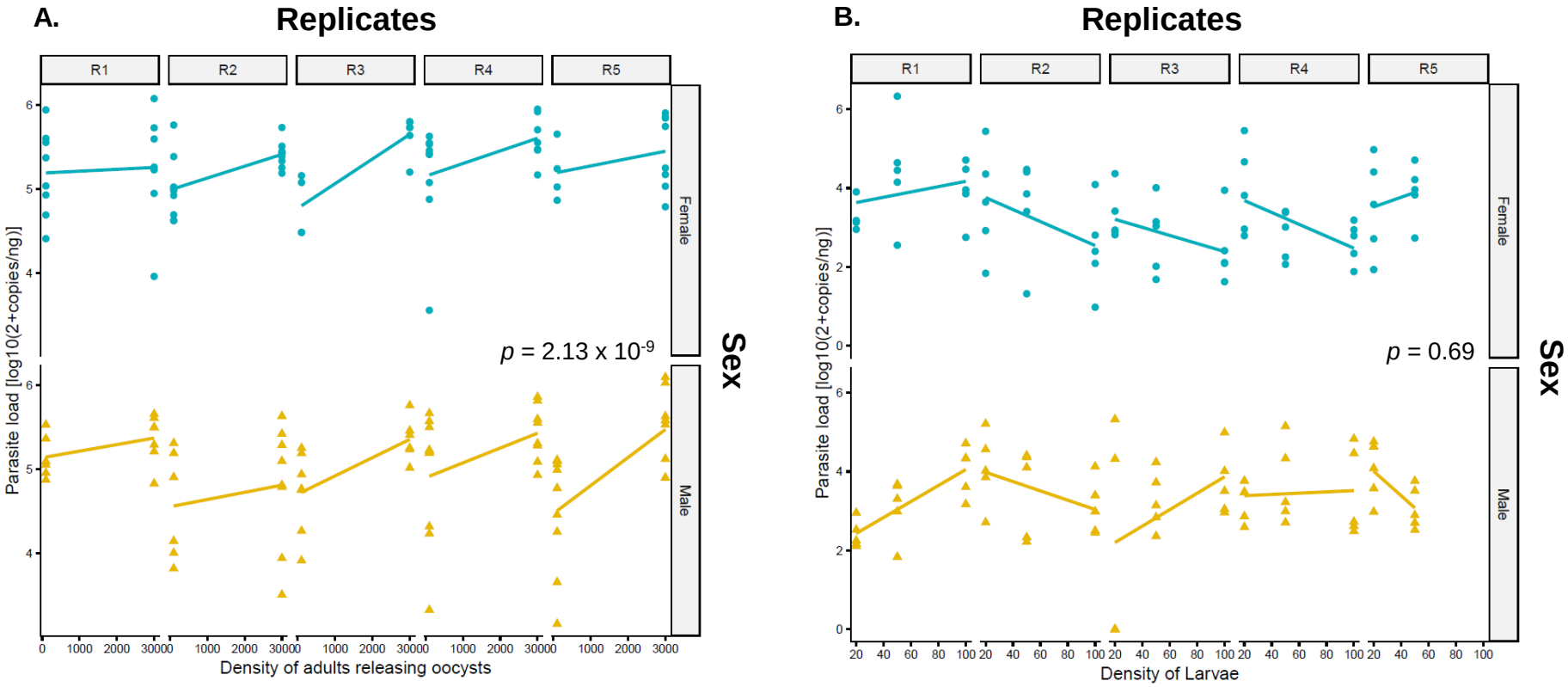

Fig. 5. Density of adults releasing oocysts positively impacts the gregarine colonization and no density dependent prophylaxis eliminates the parasite when the larval density increases. The parasite load colonizing female (blue) and male (yellow) mosquitoes that emerged, was quantified through qPCR and represented in relation with two explanatory variables (A) the density of the adult population that has released oocysts in the larval water habitat or (B) the density of infected larvae that were reared together in 100ml of water. Each experiment was replicated five times (R1 to R5). The p-value represents the significance of the explanatory variable (either the density of adults releasing oocysts or the density of larvae).

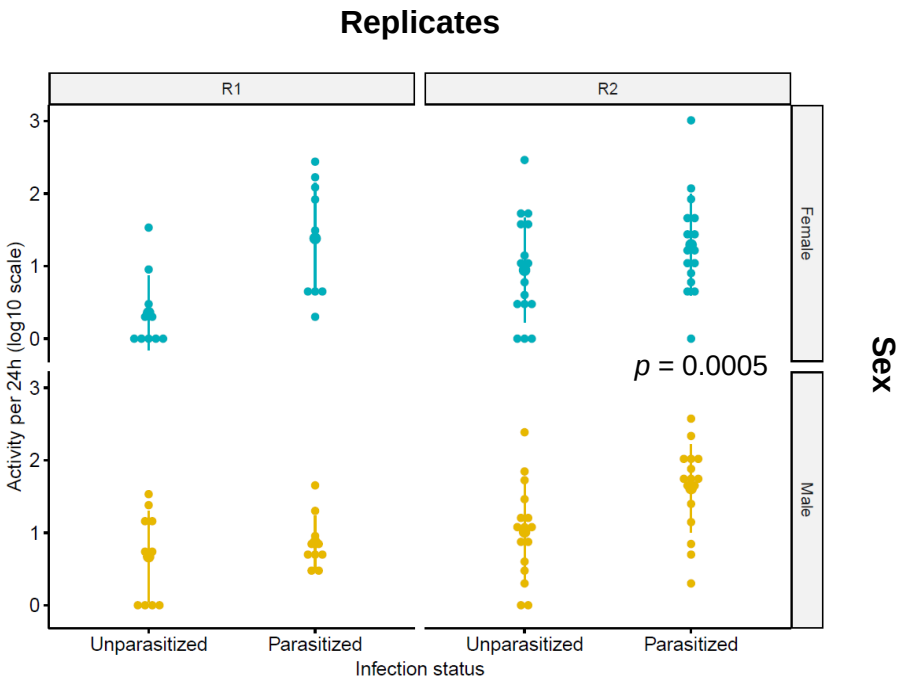

Fig. 6. *Ascogregarina taiwanensis* infection impact on mosquito flight. The flight activity of unparasitized and parasitized female (blue) and male (yellow) mosquitoes has been recorded individually using a Locomotor Activity Monitor during 24h. Each dot represent a single mosquito. The experiment was replicated twice (R1 and R2). The p-value represents the significance of the infection status.

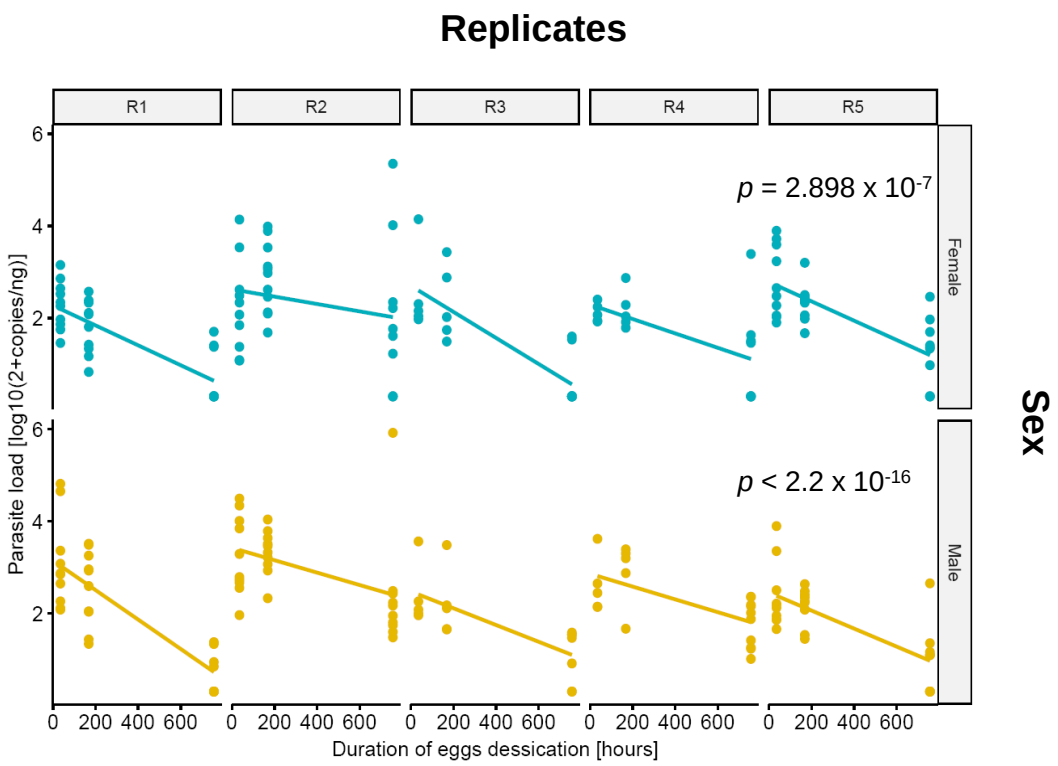

Fig. 7. Impact of egg maintenance outside of water (desiccated) on the infectivity of *As. taiwanensis*. The parasite load colonizing emerging female (blue) and male (yellow) mosquitoes was quantified through qPCR and represented in relations with the duration of dessication experienced by the emerging adults when they were eggs. The experiment was repeated five times (R1 to R5). The p-value represents the significance of the explanatory variable (duration of egg dessication) for each sex.

Table 1. Fixed factor impact on *Ascogregarina taiwanensis* prevalence, abundance and load associated with the Asian tiger mosquito natural populations and laboratory experiments associated

Response Variable	Fixed Effect	Experiments	χ^2 stat	Subsampled datasets	*p-value*	Spearman correlation index
Prevalence (GLM)	Origin		280.654		**<0.0001*** **	
	Sex		0.462		0.496585	
	Origin x Sex		25.046		**0.008976 ** **	
	Origin		198.09	Female	**<0.0001*** **	
	Origin		107.61	Male	**<0.0001*** **	
Prevalence (GLM)	Climate		1.0230e-12		1	
	Climate x Sex		-4.8772e-11		1	
Prevalence (Spearman correlation)	Age of the population			Female	**<0.0001*** **	0.5022401
				Male	**<0.0001*** **	0.3645986
Δ Prevalence (Mantel correlation)	Geographical distance			Female	0.2026	0.05730781
				Male	0.2027	0.07085295
Ascogregarina|Otu000001 abundance (GLM)	Years after introduction		0.5940		0.4409	
	Sex		0.3500		0.5541	
	Years after introduction x Sex		2.0737		0.1499	
Ascogregarina|Otu000002 abundance (GLM)	Years after introduction		19.1842		**1.187e-05 *** **	
	Sex		1.3936		0.2378	
	Years after introduction x Sex		0.4375		0.5083	
Ascogregarina|Otu000003 abundance (GLM)	Years after introduction		8.7003		**0.003182** **	
	Sex		0.0160		0.899290	
	Years after introduction x Sex		2.0092		0.156350	
Parasite load (GLM)	Adult mosquito density	Inter-individual transmission	35.85		**<0.0001*** **	
	Sex		9.03		**2.66e-3** **	
	Larval mosquito density	Density Dependent Prophylaxis	0.16		0.69	
	Sex	Desiccation resistance	4.4867		**0.03416 * **	
	Egg desiccation		26.32	Female	**<0.0001*** **	
	Egg desiccation		68.81	Male	**<0.0001*** **	
Flight activity (GLM)	Infection status	Flight activity	11.5809		**0.0007 *** **	
	Sex		0.4776		0.4894964	

Table S1. *Aedes albopictus* population used in the current study

Country	Origin site	Months of sample	Years of sample	Years after introduction	Sex	n. samples prevalence	n. samples metabarcoding	Climate	GPS coordinates
France	Saint-Priest	July	2012	0	males	8	10	Semi-continental	N : 45°41'49"
					females	28	10		E : 4°58'50'
	Saint-Priest	August	2018	1	males	24	-	Semi-continental	N: 45°43'8'
					females	28	-		E : 4°55'9"
	Oullins	July	2018	1	males	20	10	Semi-continental	N: 45°42'42"
					females	18	10		E : 4°48'2"
	Feyzin	July	2018	1	males	18	10	Semi-continental	N: 45°40'25'
					females	22	10		E : 4°51'33"
	Villeurbanne	July	2018	5	males	26	10	Semi-continental	N : 45°46'18"
						30	-		E : 4°53'24"
	Nice	August	2012	7	males	16	-	Mediteraneen	N : 43°41'60"
					females	20	10		E : 7°17'33"
	Portes-Les-Valence	July	2013	2	males	7	10	Semi-continental Mediteraneen	N : 44°52'8"
					females	31	10		E : 4°52'9'
Italy	Turin	September	2011	15	males	17	10	Mediteraneen	N: 45°4'13'
					females	13	10		E : 7°41'12"
Spain	Barcelona	July	2013	9	females	22	10	Mediteraneen	N: 41°23'6.2'
									E : 2°10'24"
USA	Atlanta	October	2018	33	males	25	10	Humid Subtropical	N : 33°44'56"
					females	24	10		O : 84°23'16"
Madagascar	Diego Suarez	NA	2009	>105	males	18	-	Humid Tropical < 900m	S : 12°16'51.24"
					females	14	10		E : 49°17'27.092"
	Toamasina	December	2010	>105	males	22	10	Humid Tropical < 900m	S : 18°8'59.64"
					females	19	10		E : 49°24'8.312'
	Tsimbaza-Park	December	2010	>105	males	13	10	Tropical > 900m	S : 18°55'40.395"
					females	25	10		E : 47°31'38.5'
Thailand	Chiang Mai	February	2019	Native	males	8	8	Tropical	N : 18° 42' 46.1"
									E : 98° 56' 17.1"
Vietnam	Hô-chi-minh	October	2012	Native	females	20	10	Tropical	N : 10°47'19"
									E : 106°42'19"
	Binh Duong	October	2012	Native	females	19	10	Tropical	N : 10°47'19"
									E : 106°42'19'
	Vüng Tau	October	2012	Native	males	25	10	Tropical	N : 10°22'26"
									E : 107°4'13"

Table S2. Fixed factor impact on the microbial divervisity associated with the Asian tiger mosquito.

Class	Response Variable	Fixed Effect	Sex	F/χ^2 stat	*p-value*	R^2
Eukaryotic microbiota	Richness (GLM)	Origin		593.93	**<2.2 x 10^{-16} *****	
		Sex		4.01	**0.0453***	
		Origin x Sex		181.48	**<2.2 x 10^{-16} *****	
	Alpha diversity (LM)	Origin		7.47	**2,738e-13 *****	
		Sex		2.81	0.09496	
		Origin x Sex		1.6457	0.12391	
	Beta diversity (permANOVA)	Origin		7.19	**0.001 *****	0.32
		Sex		1.17	0.28	0,00
		Origin x Sex		1.81	**0.001 *****	0.04
		Years after introduction	Female	4.7368	**0.001 *****	0.22
		Years after introduction	Male	4.8806	**0.001 *****	0.24
		Climate	Female	3.8823	**0.001 *****	0.08
		Climate	Male	4.7886	**0.001 *****	0.13
Bacterial microbiota	Richness (GLM)	Origin		867.33	**<2,2 x 10^{-16} *****	
		Sex		0.12	0.7319	
		Origin x Sex		162.12	**<2,2 x 10^{-16} *****	
	Alpha diversity (LM)	Origin		15.14	**<2,2 x 10^{-16} *****	
		Sex		0.78	0.38	
		Origin x Sex		19.46	**0.0087****	
	Beta diversity (permANOVA)	Origin		7.65	**0.001 *****	0.32
		Sex		7.08	**0.001 *****	0.02
		Origin x Sex		3.57	**0.001 *****	0.07
		Years after introduction	Female	3.7418	**0.001 *****	0.19
		Years after introduction	Male	4.2692	**0.001 *****	0.21
		Climate	Female	5.6248	**0.001 *****	0.11
		Climate	Male	5.9576	**0.001 *****	0.16

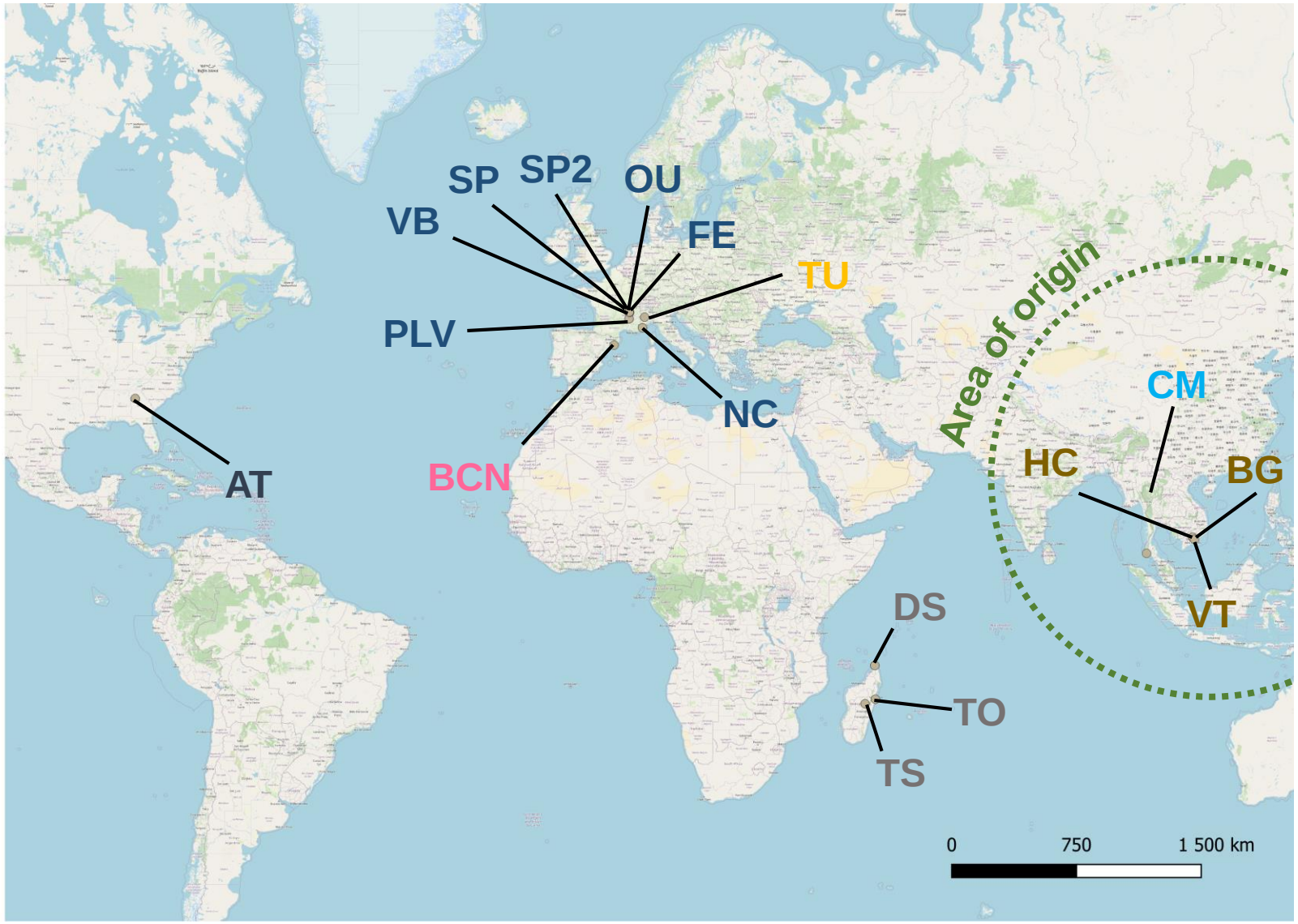

Figure S1. Worldmap representing the collection sites of mosquitoes populations used in this study.

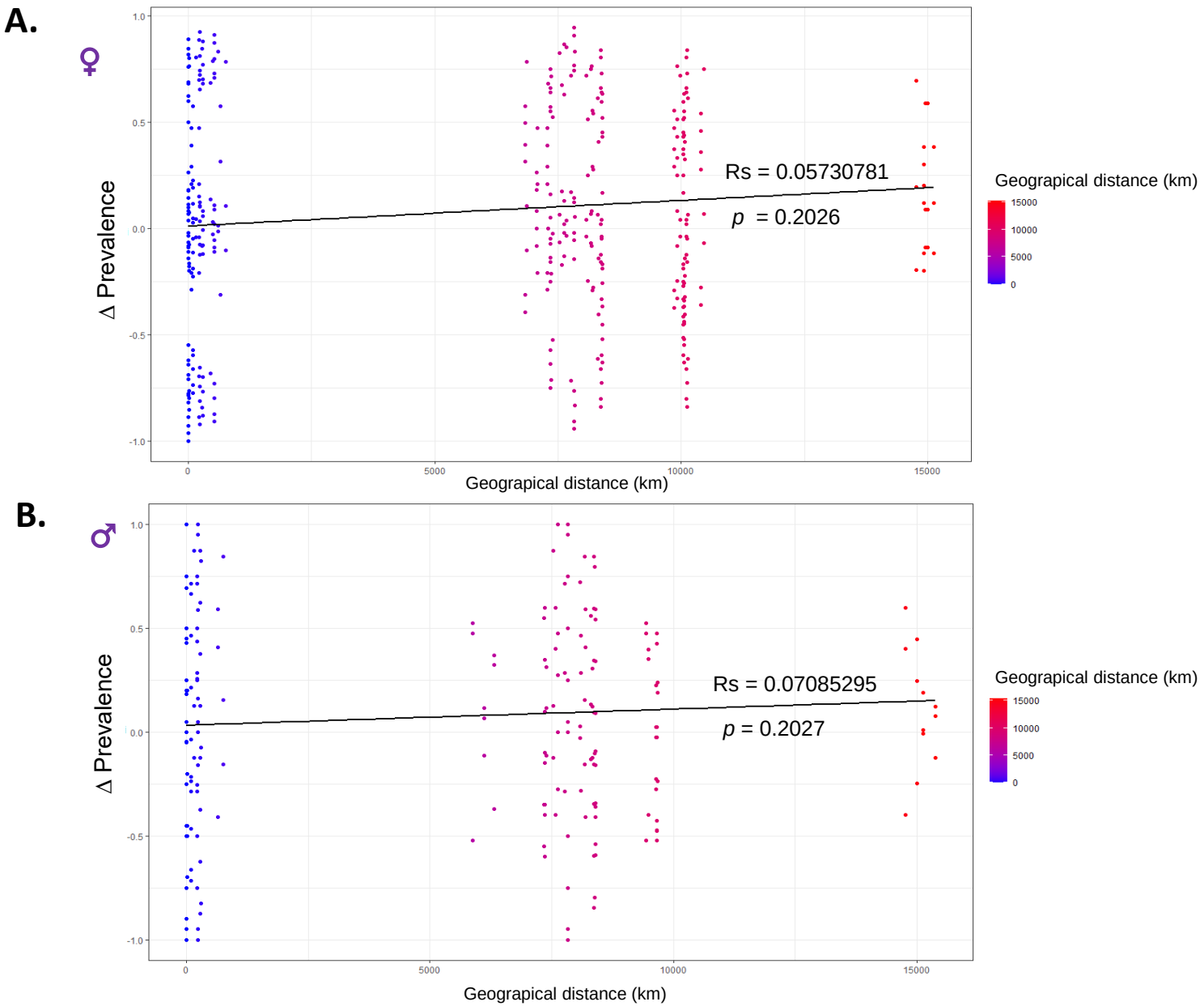

Figure S2. Prevalence variation is not significantly correlated with geographical distance. Pearson correlation analysis for (A) female and (B) male individuals did not show any correlation between prevalence variation and geographical ellipsoid distance. The points are colored with a gradient ranging from blue to red based on the geographical distance separating the two points used for the comparison.

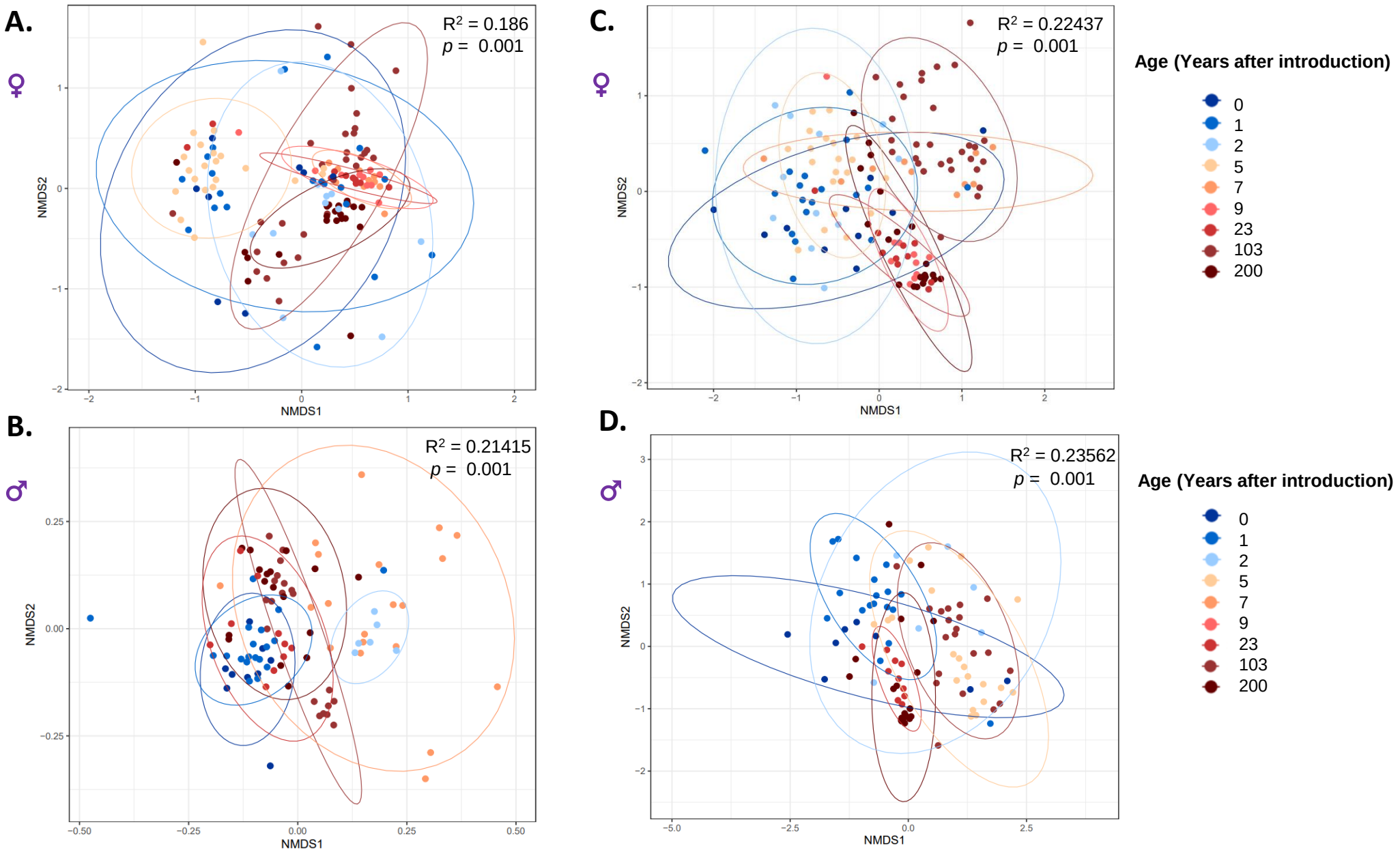

Figure S3. Microbiota dissimilarity is correlated to mosquito population years after introduction. For both (A, C) female and (B, D) male individuals a correlation have been observed between microbiota dissimilarity (bray-curtis index) and climate for both (A, B) prokaryotic and (C, D) eukaryotic microbiota. The points are colored with a gradient ranging from blue to red based on the age of each population (years after introduction). Native populations were arbitrarily set to 200 years.

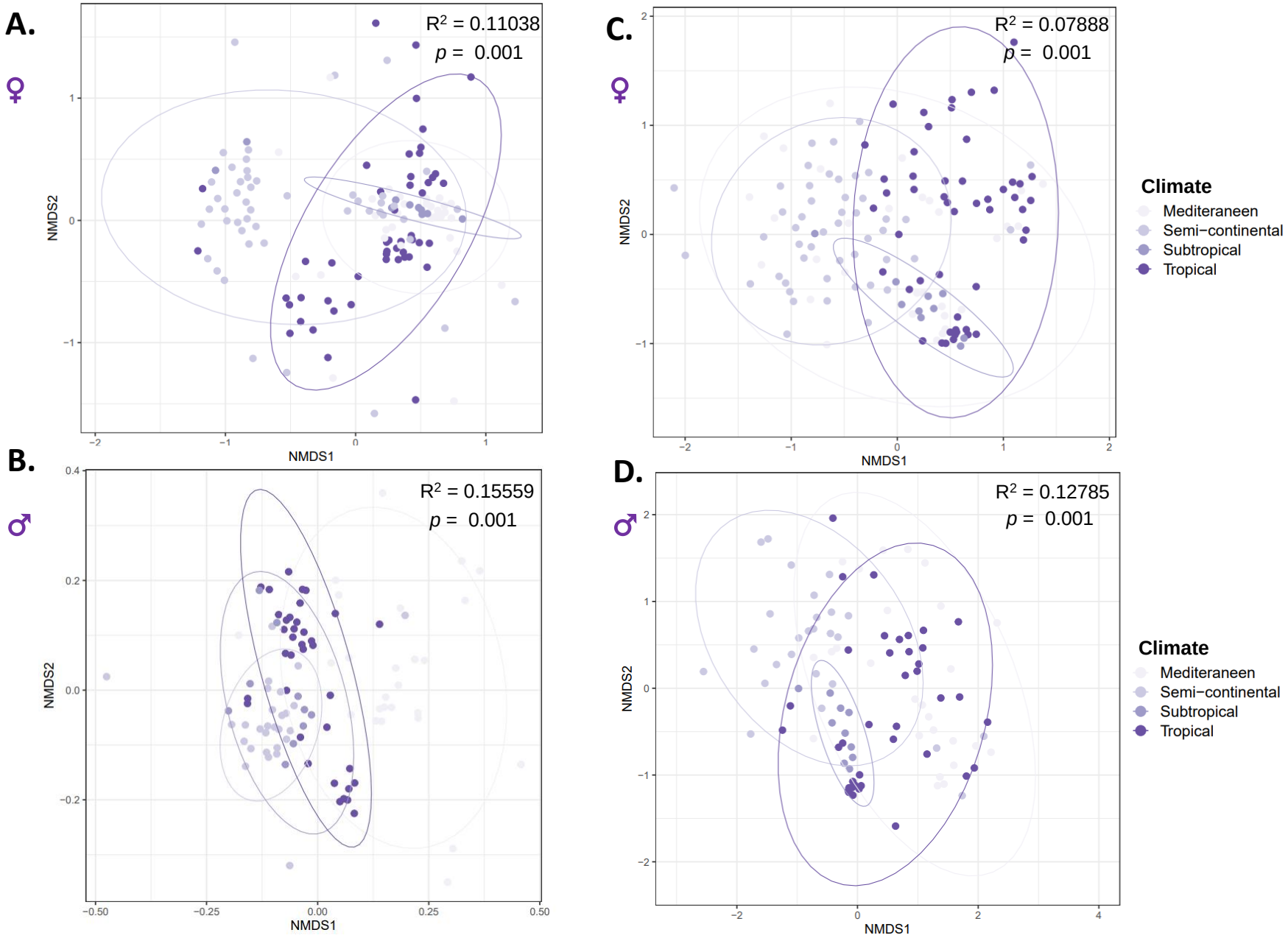

Figure S4. Microbiota dissimilarity is correlated with climate. For both (A, C) female and (B, D) male individuals a correlation have been observed between microbiota dissimilarity (bray-curtis index) and climate for (A, B) prokaryotic and (C, D) eukaryotic microbiota. The points are gradiently colored from grey to purple based on the cilmate category.

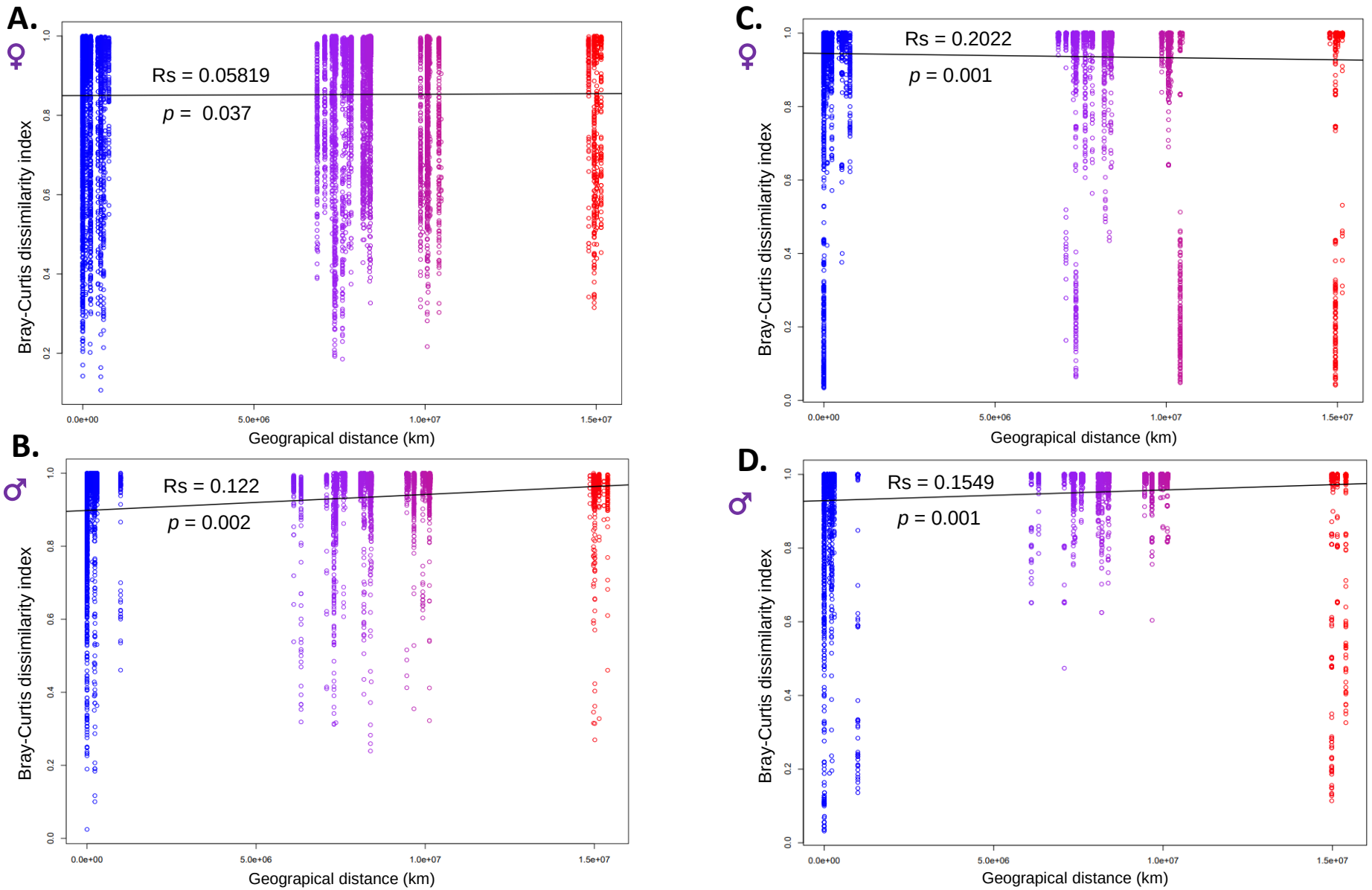

Figure S5. Microbiota dissimilarity is correlated to geographical distance. For both (A, C) female and (B, D) male individuals a correlation has been observed between microbiota dissimilarity (bray-curtis index) and geographical distance for (A, B) prokaryotic and (C, D) eukaryotic microbiota. The points are colored with a gradient ranging from blue to red based on geographical distance between both points being compared.

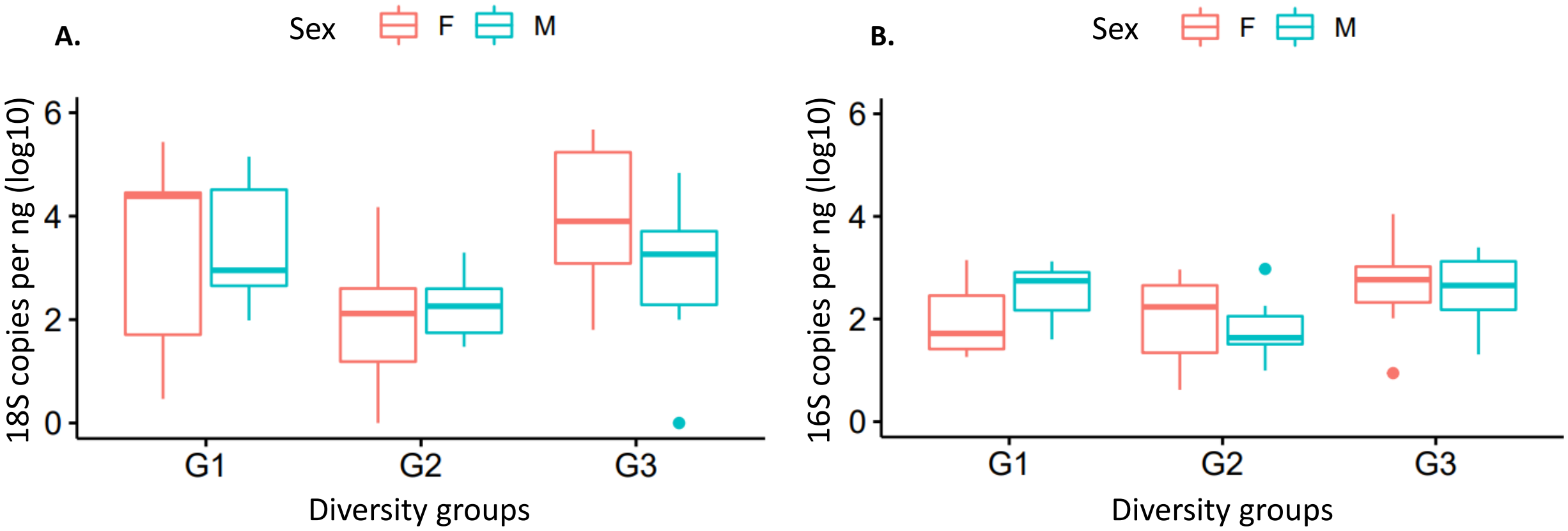

Figure S6. Quantification of microbial densities. Quantifications were performed through qPCR of the (A) 18S rDNA genes for the eukaryote community or (B) the 16S rDNA gene for the prokaryotic community. Females and males are respectively coloured in red and blue. G1 represent samples from various origins for which the *Ascogregarina taiwanensis* Otu000001 dominates the microbiota. G2 represents samples for which *As. taiwanensis* Otu000002 and Otu000003 dominates the microbiota. Finally, G3 represents samples that are not dominated by *As. taiwanensis*.

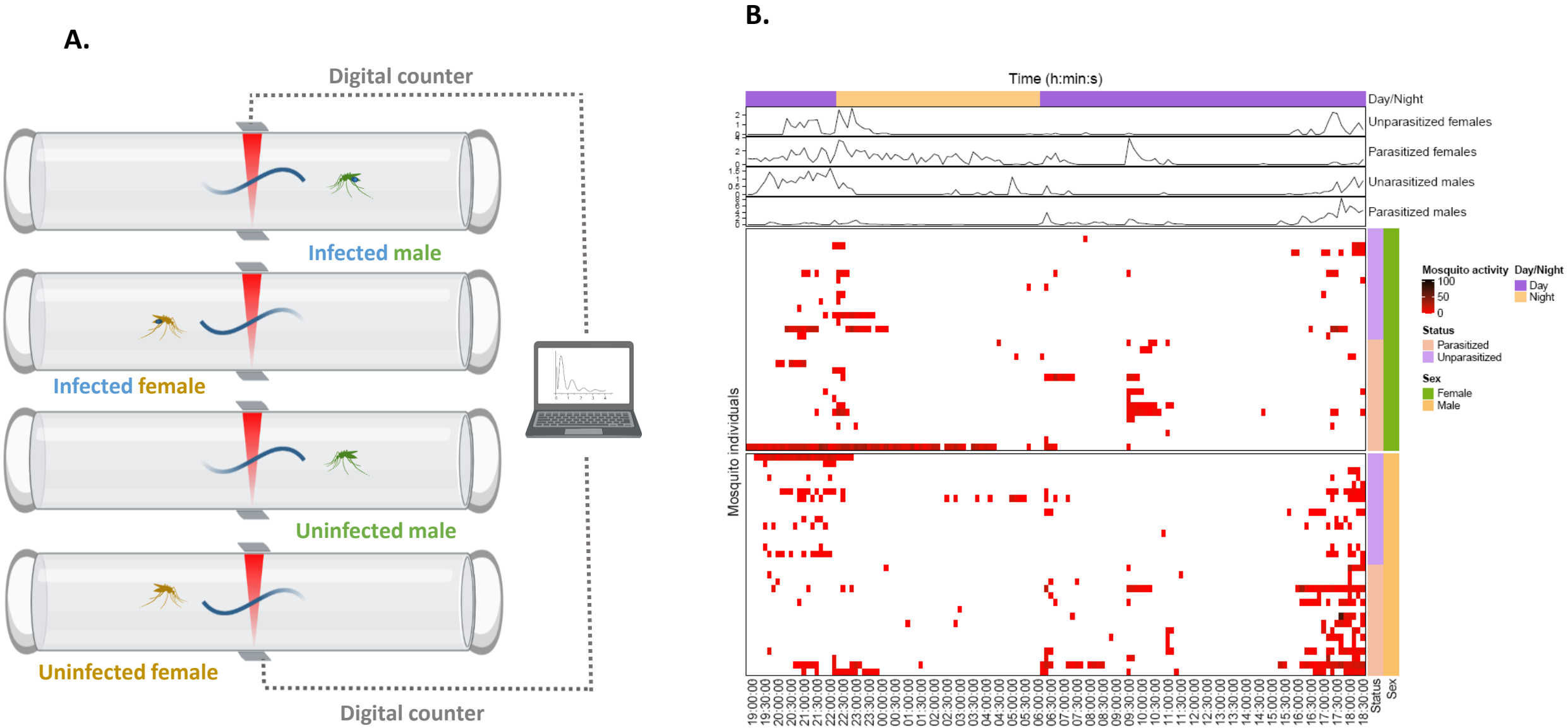

Fig. S7. Activity essays. (A) The locomotor activity monitor system, record the activity of mosquitoes through the number of time they pass through an infrared beam. (B) Activity essays were conducted for 24 hours and the mosquito activity is ploted according to the status (parasitized or unparasitized) and the sex of mosquitoes. On top of the graph, the average activity for each group is represented as well as day and night hours that were set in the insectary during the experiment.