The Search for a Model Organism for Panspermia: Examining the Effects of Vacuum and Ultraviolet Radiation Exposure on Differently Encysted Artemia Embryos

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**ABSTRACT**

Panspermia, or the idea that sources of life are distributed throughout the Universe via transportational units such as meteoroids, has become a tantalizing possibility throughout the realm of astrobiology. In this experiment, *Artemia franciscana* (brine shrimp a.k.a. Sea Monkey) embryos under different encystment states (dehydrated, hydrated-capsulated, and hydrated-decapsulated) were exposed to extreme vacuum and ultraviolet radiation conditions similar to those found in space in order to determine whether or not they could be a prototypical organism for this theory. Quantitative analyses were done by calculating *Artemia* hatch rates for umbrella, naupliar, and both umbrella & naupliar stages following treatments. Although hatch rates varied, exposure to these extreme space-like conditions did not completely impede the viability of the encysted *Artemia* studied. This suggests that encysted *Artemia franciscana* may be a model organism supporting the theory of panspermia, although the effects of encystment state during exposure to other space-like conditions besides extreme vacuum and UV radiation warrants further investigation.

**1 INTRODUCTION**

Panspermia and *Artemia* ‘Extremophilicity’

The theory that the seeds of life exist throughout the Universe and can be spread by way of travelling through space has come to be known as panspermia. In fact, this concept has been a debated topic in philosophy for nearly a millennia, with the first recorded advocate being the Greek philosopher Anaxagoras (500–428 B.C.), who argued that sources of life are extant throughout the Universe—hence panspermia’s derivation from the Greek words pan meaning ‘all’ and sperma meaning ‘seed’ [Rampelotto, 2010]. However, it was not until the mid-nineteenth century that the physician H.E. Richter first approached panspermia from a scientific standpoint. He proposed that a theoretical transportation vehicle for organisms travelling through space may be meteors [Rampelotto, 2010]. Today we have evidence that this may be possible, as studies have been done on certain hypothesized ‘seeds’ of panspermia, including the embryonic cysts of *Artemia franciscana*.

The genus of aquatic crustacean *Artemia* or brine shrimp, is known for their highly accessible and polyextremophilic encysted embryos (eggs/cysts). Consequently these cysts are an ideal subject for astrobiological panspermia experiments. Exposure to extreme environmental conditions that organisms transported through space may experience is often the interest of such investigations. Of those conditions dehydrated *Artemia* cysts have been known to preserve their embryonic vitality after cosmic ray irradiation [Gaubin et al., 1979], high ultraviolet irradiation [Tanguay et al., 2004], temperatures near absolute zero [Skoultchi & Morowitz, 1964], and vacuum conditions [Iwasaki and Kumamoto, 1976].

This experiment focuses on the effects of ultraviolet radiation-C (UVR-C) and vacuum conditions in particular, although it is novel in the fact that hydrated-capsulated and hydrated-decapsulated cysts are exposed to vacuum and UVR-C conditions in addition to dehydrated cysts. Furthermore these variables are appropriate for this experiment, considering that the theoretical transportation vessels of panspermia organisms (meteoroids from comets and asteroids) have been known to contain minute amounts of hydrating materials such as water [McSween et al., 2001; Rothery et al., 2011].

**General Hatching Characteristics of Artemia**

*Artemia* can either be born live or from dormant cysts. Favorable conditions of relatively low salt concentrations, high oxygen levels, and moderate temperatures prompt females to give birth to live young, whereas harsh conditions such as high salinity, low oxygen, and low temperatures induce the laying of encysted embryos having extremely low, almost undetectable metabolisms (diapause). These
harsh conditions are usually brought about by droughts or seasonal temperature drops, and the females lay cysts in order to ensure the survival of future Artemia generations. These cysts are the objects of interest in this study, as they were exposed to vacuum and UVR-C and shown to be variously resistant depending on capsulation status.

Once the Artemia embryos hatch from the cysts, they are encased in an embryonic sac and survive off of yolk reserves as they hang from the shell. This is known as the ‘umbrella stage’, which lasts for approximately twelve hours (Figure 1, A). After this time period, development is completed to the point in which a free-swimming young Artemia called ‘nauplius’ emerges from the embryonic sac (Figure 1, B). It is these two phases—umbrella and naupliar—which were considered hatched and therefore counted together in this study. Deeper insight into the early development of Artemia after being exposed to the space-like conditions of high vacuum and UVR-C was also thought to be gained by obtaining counts for each umbrella and naupliar phase separately.

Current Knowledge of Vacuum and UVR-C Effects on Artemia

Previous research indicates that UV radiation resistance in Artemia cysts have been attributed to their structures and certain chemical compounds. Cyst morphology consists of a yolky embryo of ~4,000 nuclei enclosed by a hard, brown hed outer capsule of lipoproteins impregnated with chitin and haematin, which may provide UV protection by phenolic tanning or melanization (Van Stappen, 1996). Figure 2 provides a visual as to what this capsule looks like.

In addition to these capsules, pigments found within Artemia cysts may also contribute to their UV radioresistance. Specifically, carotenoids (which give decapsulated cysts an orange color) are thought to play the role of antioxidants that detoxify the irradiated cysts by neutralizing free radicals generated by such irradiation (Hylander et al., 2009). Moreover there is also a possibility that trehalose, the disaccharide responsible for desiccation resistance in Artemia cysts, may also play a role in UV-radiation resistance (Nilsson et al., 2010). The differences between capsulation vs. decapsulation and possible link between desiccation and UV radioresistance were tested in this experiment through examining the hatchability of each focus group previously described after UVR-C irradiation. While literature concerning the effects of vacuum exposure on Artemia cysts in a laboratory environment is fairly limited, quite a few studies in which the cysts were taken into space reported that exposure to vacuum conditions for a few months barely affected embryonic viability (Gaubin et al., 1979; Morrison, 1977). This is no surprise given that dehydrated cysts are sometimes stored in vacuum-packed containers sold to pet stores to be used as live marine fish food. However, the Artemia cysts mechanism(s) for surviving under vacuum is not as well explored as that of their radioresistance. It was suggested that this experiment may provide insight into such mechanism(s) by taking into account the effect hydration and capsulation has on cysts exposed to vacuum conditions similar to space.

Keeping such conjectures in mind, this experiment aimed to determine whether the hatching progresses of not just dehydrated (D), but also hydrated-capsulated (HC) and hydrated-decapsulated (HD) cysts, under vacuum and UVR-C conditions is evidence for Artemia to be a model organism supporting the astrobiological theory of panspermia. It was hypothesized that, for the two distinct umbrella and naupliar hatching phases observed together and individually, there would be no significant difference in Artemia hatch rates between the control and each of the treatments (vacuum & UVR-C), as well as between cyst types (D, HC, & HD). It was also hypothesized that there is no significant interaction between the treatments and cyst types for both and each hatching phase. These hypotheses support the idea that encysted Artemia franciscana embryos are model organisms for panspermia.

Fig. 2: Cross section of an encysted Artemia embryo. (Tanguay et al., 2004) The outer layer (the capsule) covers the yolky inside, in which the large gray circles are lipids.

2 MATERIALS AND METHODS

Cyst Preparations

Cysts were obtained from Brine Shrimp Direct (Premium Grade, 90% hatch rate). Five replicates comprised each of the dehydrated (D), hydrated-capsulated (HC), and hydrated-decapsulated (HD) groups. For the D cysts, 0.040 g (~10,000 cysts) were weighed out onto filter paper in a petri dish. For the HC cysts, 0.040 g were hydrated in a 50 mL falcon tube overnight in a refrigerator (2°C) in a saline solution (14.8 mL, 30 ppt) and then filtered out onto a filter, which was placed in a petri dish. The HD cysts underwent the same procedure, except decapsulation was done. This procedure consisted of 9.9 mL of 6% NaClO being added to the 50 mL falcon tube holding the cysts, which was then put on a solution shaker for 20 minutes. The falcon tube was kept on ice throughout the duration of this process to suppress the encysted Artemia embryos’ metabolisms. The cysts were then filtered out with cold deionized (DI) water for 3 minutes, soaked in a chilled...
vinegar solution (32 mL DI water + 2 mL white distilled vinegar), and rewashed with DI water for 3 minutes again until the smell of bleach and vinegar was gone. These D, HC, and HD groups were then exposed to control, UVR-C, and vacuum treatments. All cysts on their respective petri dishes were spread in a monolayer before being exposed. See Table 1 for a summary of these groups and treatments. Each treatment contained approximately 10,000 cysts, giving 150,000 cysts for each condition, for a total of 450,000 cysts in this study.

Table 1. Summary of total replicates and cyst numbers.

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>HC</th>
<th>HD</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Vacuum</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>UVR-C</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>total</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>45</td>
</tr>
</tbody>
</table>

Vacuum and UVR-C Treatments

For UVR-C all groups were exposed to a wavelength of 254 nm at an intensity of 706 µW cm⁻² for 20 minutes using a UVP Benchtop 3UV Transilluminator. For vacuum, all groups were exposed to a vacuum of 0.10 mtorr (0.013 Pa) for 6 hours under a 12 inch diameter glass bell jar using a Cenco-Megavac Vacuum Pump. Each treatment exposure was done one day at a time, with counting executed in between treatment days. See Figure 3 below.

Incubation and Counting Cysts

Each replicate of cysts was washed off the filter into a 250 mL Erlenmeyer flask using saline (100 mL, 30%) and left to incubate with similar aeration at 76 °C under a light of 40 W for 24 hours (Figure 3). After that time period, 25 drops of iodine was added to prevent further hatching and kill the *Artemia* (to make them countable). Counting was done under a dissecting microscope. One ml from each Erlenmeyer flask was then taken, and the average number of *Artemia* for three counts was multiplied by the starting volume ($V_o$) to get the number hatched for each flask. This was then divided by the starting number of cysts ($1 \times 10^4$) to find the percentage of cysts that hatched:

$$\text{% Hatched} = \frac{\text{Average} \times V_o}{1 \times 10^4}$$

For statistical analysis between the control and treatments, cyst types, and interactions between treatments vs. cyst type a 2-Factor analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$ was done in Excel using the data calculated with Equation 1.

3 DATA AND RESULTS

Cyst Morphologies After Exposure Treatments

All cyst types re-dehydrated after vacuum exposure, whereas after UVR-C exposure the HD and HC cysts stayed hydrated. Furthermore, HD cysts retained their orange-yellow decapsulation colors (Figure 4). Also, orange-yellow residue was left behind on the filters the cysts were placed on during vacuum exposure (Figure 5). The origin of this residue is discussed in Section 4. The filters for the other treatment exposures did not exhibit any residue and therefore were not included.

Fig. 4: Visual appearance of D, HC and HD cysts after treatments

Fig. 5: Residue after vacuum treatment: the arrows indicate orange-yellow residue left over on the HC and HD filters after vacuum. D filters did not contain any residue.

Counting Data

Umbrella+Naupliar The percentages of umbrella-naupliar *Artemia* hatched are presented in Figure 6. Although rates varied, all groups exhibited hatching. Numerical values of these data are tabulated in Table 2.
Vacuum exposure did not significantly alter survivability for umbrella-naupliar *Artemia* (2-way ANOVA, $F_{0.05, 1.14} = 0.00555$, $P = 0.941$); however, UVR-C did in that it greatly decreased the number hatched (2-way ANOVA, $F_{0.05, 1.14} = 7.55$, $P = 0.0112$). Furthermore hatch rates among cyst types subjected to vacuum and naupliar *Artemia* did not markedly vary (2-way ANOVA, $F_{0.05, 2.13} = 0.949$, $P = 0.401$), whereas those for cyst types exposed to UVR-C did indicate difference (2-way ANOVA, $F_{0.05, 2.13} = 10.802$, $P = 0.000451$). Finally, it is clear from these patterns that for umbrella-naupliar there is no significant interaction between vacuum exposure and cyst type (2-way ANOVA, $F_{0.05, 2.13} = 0.949$, $P = 0.000551$), though there is a substantial interaction between UVR-C treatment and cyst type (2-way ANOVA, $F_{0.05, 2.13} = 10.425$, $P = 0.000551$).

### Table 2. Totals of umbrella-naupliar *Artemia* hatched.

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>HC</th>
<th>HD</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6958±814</td>
<td>8024±1279</td>
<td>7607±819</td>
<td>22589±2912</td>
</tr>
<tr>
<td>Vacuum</td>
<td>8024±1376</td>
<td>879±460</td>
<td>5988±1776</td>
<td>22802±3612</td>
</tr>
<tr>
<td>UVR-C</td>
<td>6991±1523</td>
<td>873±470</td>
<td>407±120</td>
<td>16128±2113</td>
</tr>
<tr>
<td>total</td>
<td>21973±3713</td>
<td>2554±2146</td>
<td>14002±2715</td>
<td>61519±8637</td>
</tr>
</tbody>
</table>

*Nauplius* The grouped bar graph displaying the percentages of nauplius *Artemia* hatched in Figure 8 show that—although rates still vary—all groups exhibited hatching. Numerical values of these data are also displayed in Table 3.

Vacuum exposure did not significantly alter hatch rate of naupliar *Artemia* (2-way ANOVA, $F_{0.05, 1.14} = 0.922$, $P = 0.346$); however, UVR-C did in that it greatly decreased the number hatched (2-way ANOVA, $F_{0.05, 1.14} = 7.18$, $P = 0.0130$). Furthermore hatch rates among cyst types subjected to vacuum and UVR-C individually for naupliar *Artemia* did not markedly vary (2-way ANOVA, $F_{0.05, 2.13} = 0.0810$, $P = 0.922$ for vacuum; 2-way ANOVA, $F_{0.05, 2.13} = 1.87$, $P = 0.176$ for UVR-C). Finally, it is clear from these patterns that for nauplius there is no significant interaction between vacuum exposure and cyst type (2-way ANOVA, $F_{0.05, 2.13} = 0.949$, $P = 0.0660$), though there is a substantial interaction between UVR-C treatment and cyst type (2-way ANOVA, $F_{0.05, 2.13} = 10.43$, $P = 0.000500$).

The interaction between which cyst type(s) with vacuum and UVR-C exposures, as well as location of the difference(s) between the three cyst types in the hatch rate (see Tables 2 and 3 for vacuum and UVR-C, are unknown since another statistical test would need to be implemented to determine such inner differences. But because

### Table 3. Totals of umbrella *Artemia* hatched.

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>HC</th>
<th>HD</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1032±385</td>
<td>3956±2588</td>
<td>7384±203</td>
<td>12372±2770</td>
</tr>
<tr>
<td>Vacuum</td>
<td>3346±342</td>
<td>2955±510</td>
<td>2411±703</td>
<td>8712±1555</td>
</tr>
<tr>
<td>UVR-C</td>
<td>3997±1106</td>
<td>1929±1184</td>
<td>343±102</td>
<td>6269±2392</td>
</tr>
<tr>
<td>total</td>
<td>8375±4234</td>
<td>8840±6374</td>
<td>10138±2253</td>
<td>27353±6717</td>
</tr>
</tbody>
</table>
it was unplanned (and therefore would increase the likelihood of a Type I error) an additional statistics test was not performed. In addition, assumptions of these tests were violated in that not all variables were normally distributed with equal variances, although the ANOVAs performed are still informative considering that this study design was balanced, as previously seen in Table 1.

![Graph showing naupliar Artemia hatch rates for each treatment and cyst type.](image)

Table 4. Totals of naupliar Artemia hatched.

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>HC</th>
<th>HD</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5926±953</td>
<td>4068±719</td>
<td>6869±955</td>
<td>16863±2627</td>
</tr>
<tr>
<td>Vacuum</td>
<td>4906±1408</td>
<td>5925±823</td>
<td>3577±1241</td>
<td>14408±3472</td>
</tr>
<tr>
<td>UVR-C</td>
<td>2994±1579</td>
<td>6923±1329</td>
<td>64±39</td>
<td>9981±2947</td>
</tr>
<tr>
<td>total</td>
<td>13826±940</td>
<td>16916±2871</td>
<td>10510±2235</td>
<td>41252±9046</td>
</tr>
</tbody>
</table>

4 DISCUSSION

In this experiment, the null hypotheses expected of no significant difference between hatch rates of the control and each of the treatments (vacuum & UVR-C), cyst types (D, HC, & HD), and no interaction between the two were tested for both and each individual umbrella and naupliar phase. These hypotheses would support the idea that Artemia are prototypical organisms for panspermia, and a summary of whether they were rejected or accepted will now be discussed in relation to Table 4.

Hypothesis 1 (H1)

In this study it was discovered that there are no significant differences in Artemia hatch rates between the control and vacuum groups for umbrella-naupliar, umbrella, and naupliar. This may be due to vacuum exposure resulting in just re-dehydration of the cysts (Figure 4). As such, this study suggests that re-dehydration does not significantly alter general Artemia cyst viability, as supported by the findings of Gaubin et al. [Gaubin et al., 1979] and Morrison [Morrison, 1977] for desiccated cysts in space. Nonetheless, effects of encystment type during vacuum treatment will subsequently be discussed in Section 4.3 in addition to any hypothetical implications vacuum exposure may have on encysted Artemia embryos travelling through space.

Furthermore, there is no significant difference between the control and UVR-C exposure hatch rates for umbrella, while for umbrella-naupliar and naupliar there is a significant difference between hatchability of the control and those cysts exposed to UVR-C. Both umbrella-naupliar and naupliar exhibited a decrease in number of Artemia hatched after UVR-C exposure (Tables 2, 4). This may be due to UVR-C inducing cytogenic damage in organisms like Artemia [Epel et al., 1999]. Such cytogenic damage includes pyrimidine dimers, which create premutagenic DNA lesions that arrests replication via deformation of the double helix structure and subsequent polymerase inhibition. This leads to cell expiration, and may ultimately result in death of the organism. Therefore, it may be assumed that the survivorship of encysted Artemia embryos transported from one world to another through space might decrease due to UVR-C irradiation.

Hypothesis 2 (H2)

This experiment shows that there is no significant difference between hatchability of cyst types after vacuum exposure for umbrella-naupliar and naupliar; however, there is a significant difference between that of cyst types for umbrella phase post-vacuum. At first glance it seems that the former resulting hypothesis of insignificant difference may be due to vacuum treatment just rehydrating every cyst type, as discussed previously. Nonetheless this supposition proves to be inadequate for explaining cyst viability when taking into account the latter resulting hypothesis that hatch rates between cyst types differed significantly after vacuum exposure for umbrella phase Artemia. Which specific cyst types contain the notable difference(s) in hatch rate among the post-vacuum trend HD < HC < D (Table 3) is unclear (as explained in Section 3.2), though some clues may be gleaned from the orange-yellow colored spot-like residues left behind on the HD and HC filters under the cysts after vacuum treatment (Figure 5). This possibly could have been some of the yolk from inside of the encysted embryos, which suggests that yolk leakage after vacuum exposure might explain any significant difference in the survivorship trend HD < HC < D for umbrella phase. How exactly encystment type may affect hatch rate during vacuum exposure will be discussed further in Section 4.3, along with any conjectural implications such exposure might have on encysted Artemia embryos traversing space.

Additionally there is no significant difference in Artemia hatch rates between cyst types after UVR-C exposure for naupliar phase, whereas there is a significant difference in Artemia hatchability for umbrella-naupliar and umbrella following UVR-C treatment. Again, which specific cyst types contain the notable difference(s) among the survivorship trend HD < HC < D for umbrella-naupliar and umbrella after UVR-C exposure is unclear (as discussed in Section 3.2), although it is quite evident that hatch rates for HD were much less than D following UVR-C exposure in both cases (Tables 2 and 3). If the significant difference in hatching probability does at least lie between the HD and D cyst types, then it may provide evidence for a link between desiccation and UV radioreistance—a mechanism maybe involving trehalose as conjectured by Nilsson.
Further cause for a possible difference between HD and D cyst types—and perhaps among even HD and HC—may stem from capsulation status, as will be discussed in section 5 along with how any interaction between cyst type and UVR-C in space might affect hatch rates and therefore survivability of encysted Artemia embryos.

Table 5. Outcomes are based on 2-way ANOVA results shown in parentheses. There are three null hypotheses (H₁, H₂, H₃) that support the idea that Artemia cysts are prototypical specimens for panspermia.

<table>
<thead>
<tr>
<th></th>
<th>H₁</th>
<th>H₂</th>
<th>H₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>umbrella-naupliar</td>
<td>✓ (0.940) ✓ (0.401) ✓ (0.466)</td>
<td>× (0.0112) × (0.000451) × (0.000551)</td>
<td></td>
</tr>
<tr>
<td>umbrella</td>
<td>✓ (0.0660) × (0.0200) × (0.0350)</td>
<td>✓ (0.795) × (0.0210) × (0.0220)</td>
<td></td>
</tr>
<tr>
<td>naupliar</td>
<td>✓ (0.346) ✓ (0.922) ✓ (0.6660)</td>
<td>× (0.0130) × (0.176) × (0.000500)</td>
<td></td>
</tr>
</tbody>
</table>

The symbol ✓ means failed to reject the hypothesis, while × means rejected. Blue represents the control vs. vacuum comparisons, and magenta is control vs. UVR-C.

Hypothesis 3 (H₃)

It was observed that there is no significant interaction between the vacuum treatment and cyst types for umbrella-naupliar and naupliar phase, but for umbrella there is. As previously discussed, hatch rates for umbrella after vacuum exposure are as follows: HD < HC < D (Table 5). Moreover, the hatch rates for naupliar following vacuum treatment are borderline insignificant in terms of statistical values (Table 5)—if a larger sample size were to be used than the one in this study, then results for naupliar may reveal a significant interaction between the cyst types and vacuum treatment, with a similar hatchability trend to post-vacuum umbrella. This trend may be due to the supposition that embryos might have been more susceptible to the environment if their cyst capsules were altered during hydration or fully removed by decapsulation. This is once again further supported by the yellow-orange spots that were observed on the HD and HC filters on which the cysts were altered during hydration or completely removed by decapsulation. If this was yolk that leaked into the incubation flasks (Figure 5). If this was yolk that leaked into the incubation flasks (Figure 5).

5 CONCLUSIONS

In this experiment, the aim was to determine whether the viability of not just dehydrated (D), but also hydrated-capsulated (HC) and hydrated-decapsulated (HD) cysts, under vacuum and UVR-C conditions is evidence for Artemia to be a model organism supporting the astrobiological theory of panspermia. The three main hypotheses expected (Table 5)—that also supported the idea of encysted Artemia embryos being a prototypical organism for panspermia—were either rejected or not in a variable manner throughout the different groups counted. Nonetheless, while the statistical tests of this study imply that UVR-C and vacuum exposure does alter the viability of differently encysted Artemia embryos, it did not completely prevent them from hatching. The verity that all groups of cyst embryos yielded hatched Artemia in this study shows that this organism may survive a trip through space-like conditions of vacuum and UVR-C radiation. These findings, along with previous studies on cosmic ray irradiation and absolute zero temperature exposure, suggests that encysted Artemia might be an ideal organism for the theory of panspermia. The outcomes of this experiment suggest that any future work with Artemia cysts in space should be done with expectations of cyst loss. Such future work may include examining the effects of encystment state during exposure to other space-like conditions besides extreme vacuum and UV radiation, as well as the UVR-C resistant properties of the capsule surrounding Artemia cysts. Furthermore, as this study’s results did not provide any substantial insight into the effects UVR-C and vacuum have on the early developmental stages of Artemia, additional 2-way ANOVA are called for to inspect this secondary hypothesis. Such an inspection may be astrobiologically beneficial as it might provide deeper insight into the already promisingly high probability that encysted Artemia franciscana may be a model organism supporting the theory of panspermia.

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