

This supplementary information contains a table (ST-1), a dataset summary (S2.1), and discussion of bias analyses (S2.2), previous spatial analyses (S2.3), size distribution analyses (S2.4), and reproductive modes (S2.5).

## ST-1

Surface	CSR	$y$	$x$	$xy$	$h$
D	NA	0.0136	0.0103	0.0125	0.0000
E	NA	0.0085	0.0080	0.0084	0.0000
H14	0.0001	0.0001	0.0107	NA	NA
D retrodeformed	0.0011	0.0059	0.0003	0.0000	NA
E retrodeformed	0.0166	0.0339	0.0132	0.0000	NA

**Supplementary Table 1:** Table of  $p$ -values for Kolmogorov Smirnov tests of inhomogeneous Poisson models for the ‘D’, ‘E’ and H14 surfaces.  $p = 1$  corresponds to a perfect model fit – the spatial distributions depend exactly on the covariant.  $x$  is North to South (parallel to strike),  $y$  is East to West (parallel to dip),  $xy$  is the distance from the South-East corner and  $h$  is the distance above the troughs of the tectonic ripples.

## S2.1 Dataset summary

A total of 5538 specimens were recorded in this study, 2977 from the 'E' surface (63.5m<sup>2</sup> retrodeformed), 1402 from the 'D' surface (62.0m<sup>2</sup> retrodeformed) and 1159 from the H14 surface (26.7m<sup>2</sup> unretrodeformed). *Fractofusus* was the dominant taxon on both the 'D' surface (76.3% of specimens documented), and the H14 surface (98.4% of specimens). On the 'E' surface, *Fractofusus* is the most common taxon, comprising 38.4% of the total specimens present. On the 'E' surface, 1140 *Fractofusus* were mapped, with an average density of 20.7/m<sup>2</sup> (Fig 1A). On the 'D' surface, 1070 *Fractofusus* were mapped, with a density of 22.4/m<sup>2</sup> (Fig 1C). *Charniodiscus* (11.0%), *Primocandelabrum* (9.1%), *Bradgatia* (1.1%), *Thectardis* (1.3%), Lobate Discs (5.4%), Ivesheadiomorphs (7.9%) and Charniids (2.6%), were also present on the 'E' surface, along with 'other taxa' (3.9%) and 'holdfast discs with stems' (18.1%). On the 'D' surface, *Bradgatia* (9.9%), *Pectinifrons* (7.6%), Charniids (1.7%) and Ivesheadiomorphs (1.8%) were present ('other taxa' 1.7%). On the H14 surface there were three unidentifiable frondose organisms (0.3%), one possible *Thectardis* (<0.1%) and 14 Ivesheadiomorphs (1.2%).

On the H14 surface, 1214 *Fractofusus* were mapped, with a density of 42.8/m<sup>2</sup> (Fig. 1D). Analysis of the size distributions of the H14 specimens (Methods 1.8) revealed three size classes: a small size class (<5.5 cm in length; 3.7±0.08cm; 45.6% of *Fractofusus* specimens), an intermediate size class (5.5–11.0 cm; 7.6±0.28cm; 25.5% of *Fractofusus*), and a large size class (11.0–41.6 cm; mean length of 17.8±2.94cm; 28.8%).

## 2.2 Biases

There are three principal sources of bias that could potentially influence our results: those introduced during data collection (observer bias), during recent weathering (erosional bias), and during the assembly of the original populations (time-averaging).

### *Observer bias*

Identification differences due to varying natural light conditions were investigated using a Mann-Whitney test. We found no significant difference in fossil density between optimal dry weather with good sunlight, and wet weather with low light ( $p = 0.2426$ ).

Given our unambiguous demonstration of clusters of clusters (of *Fractofusus*) on both the 'E' and H14 surfaces, it is curious that these have not been detected in the field. At least in part, this derives from the large size of individual clusters (the largest contain 12 second-order clusters of 3 specimens) combined with the limited field of view from ground-based observations. Such scaling issues highlight the importance of statistical analysis for resolving spatial patterns (Extended data Fig. 5).

### *Erosional bias*

Erosional bias was tested using inhomogeneous Poisson models. Specimen densities were not found to depend directly on any of the four covariates, likely because specimens and their outlines are identifiable even when under a (thin) veneer of ash<sup>1</sup> ( $p < 0.01$ ); Supplementary Table 1); thus, the homogeneous Poisson model is a suitable null model for aggregation modelling.

The taxonomic group spatial distributions on the 'E' surface provide further evidence of the lack of significant bias from modern erosional effects, such as differential erosion of ash. If the fossils (or a taxon) were randomly distributed, but masked by patchily distributed

ash, the resulting aggregation patterns would be of the same spatial scale as the ash patches; as such, would be expected to yield similar aggregation patterns across all taxa. Of the five taxa on the 'E' surface that are aggregated, however, each exhibits a distinct aggregation pattern and different scales of clustering. If there is a bias in terms of fossil exposure, it is necessarily small and has no statistically significant effect on measured distributions.

### *Time averaging*

Soft-bodied organisms such as rangeomorphs are highly unlikely to accumulate as time-averaged assemblages in the manner of shelly fossils.<sup>56</sup> Time-averaging might nonetheless occur via 'secondary succession',<sup>57</sup> where a new cohort of organisms settles within a previously established (or recently deceased) community, imparting a mixed signal to the resulting spatial distributions. The likelihood of such succession can be assessed by considering the spatial distributions in their geological context. Assuming that secondary colonizers will be derived from water-borne propagules, we would expect these to exhibit random bedding plane distributions and to comprise the smaller specimens of a community. On the H14 surface, however, small specimens are highly aggregated and clustered around larger specimens. Such clustering could conceivably occur if there was a strong environmental variable influencing both the primary and secondary colonizers; however, our PCF analysis found that the small size-class clusters were not related to environmental processes (Extended data Tables 1–4), so this hypothesis can be robustly rejected. The lack of size data precludes such analysis of the 'D' and 'E' surfaces, but the similarity of the overall spatial patterns (Extended data Table 3), argues convincingly against any significant effect from secondary succession.

Secondary community succession can alternatively be recognised by the presence of a high proportion of effaced, partially decayed specimens from an earlier community.<sup>57,58</sup> No

*Fractofusus* specimens on the 'D', 'E' and H14 surfaces appear to be effaced in this manner, nor was there evidence on any of the studied surfaces for time averaging due to sub-lethal sedimentation events.<sup>59</sup> Indeed, if significantly effaced specimens had been identified, they would have been included within the ivesheadiomorph grouping,<sup>58</sup> rather than within *Fractofusus*. This geological evidence further points to a single, established community with no secondary community succession on all three surfaces.

## 2.3 Previous spatial and size-distribution analysis of Mistaken Point communities

### *Previous Spatial Analysis*

The nearest neighbour analyses previously applied to single taxonomic groups of Mistaken Point fossils was able to distinguished random vs. non-random spatial patterns<sup>10</sup>, but with no quantification of the results. By quantifying the details of spatial distribution, PCF offers a fundamentally more resolved account of the underlying behaviour. Our PCF analysis, for example, demonstrated that the small size class of *Fractofusus* on the H14 surface was six times more aggregated than would be expected from a random distribution (Fig 2c).

Differential scaling is also an issue for nearest neighbour analysis because of the magnitude isn't clearly recorded. Although the nearest neighbour distribution of *Fractofusus* specimens on 'E' surface shows a clear aggregation under 0.2m, PCF of the same data reveals two distinct spatial scales of aggregation (Extended data Fig. 4b,f) Moreover, nearest neighbour analysis is likely to miss aggregations on heterogeneous backgrounds (where distribution is random at one scale but not at others), and cannot distinguish different types of aggregation process (e.g. heterogeneous Poisson vs Thomas Cluster process); by contrast, our PCF analysis identified three different types of aggregation patterns on the 'E' surface, and two on the H14 surface. By finding the best-fit model for each non-random distribution, these quantitative techniques also allow statistical comparison of different taxa and bedding planes; for example, our analysis confirms that the aggregations observed on the 'E' surface can be modelled by the same process as the aggregations on the H14 surface. Finally, nearest neighbour analysis is limited to the maximum distance between two specimens: if all *Fractofusus* are found within 0.2m of each other, then analysis is limited to under 0.2m, and any larger-scale patterns will go undetected (vs. the range of PCF analysis which can extend to kilometers<sup>8,18</sup>)

*Previous Size-based analysis*

Darroch et al.<sup>7</sup> carried out size-frequency distribution analysis on Mistaken Point taxa including *Fractofusus*, using the results to comment on the relative seasonality of reproduction. These data were interpreted as representing single size-based populations with continuous (aseasonal) reproduction. By contrast, our size-frequency analyses of *Fractofusus* on the H14 surface identified three populations, pointing to discontinuous reproduction (with very strong significance; Methods section: Size classes). Both waterborne propagule dispersal and stolon-like reproduction can occur continuously or discontinuously, which may be reflected in these two conspicuously different (but not necessarily incompatible) results. In any event, the determination of reproductive seasonality does not resolve the particular mechanism of that reproduction.

## 2.4 Size distribution analysis

The mark correlation function shows that there was dependency between *Fractofusus* sizes on H14 surface at small distances ( $<0.20\text{m}$ ,  $p_d = 0.01$ ), and that the dependency was positively correlated: *Fractofusus* specimens that are closer together than  $0.20\text{m}$  are smaller than might be expected by random chance (Extended data Fig. 4a). In addition to using BIC to assess different size-based population models, size classes may alternatively be defined by splitting the population into groups of equal number, equal length range, or by visual inspection of cumulative frequency plots. Very similar clustering patterns were observed for each method of size class definition,

## 2.5 Ecological successions

Clapham et al.<sup>10</sup> have suggested that the ‘D’ and ‘E’ surfaces represent two stages of an ecological succession, where the low taxonomic diversity on ‘D’ surface represents an early, relatively undeveloped phase of the more diverse community structure seen on the ‘E’ surface. Such interpretation is supported by the spatial distribution patterns of *Fractofusus* which exhibit just two generations on the ‘D’ surface and three generations on the ‘E’ surface. Size analysis of the H14 surface further corroborated this point: the PCF of the largest size class on the ‘H14’ surface and the PCF of the ‘D’ surface *Fractofusus* population can both be described by the same model ( $p_d=0.65$ , Extended data Fig. 4e) suggesting that they derive from the same underlying processes. As such the ‘D’ surface appears to represent the same stage of population development as represented by the largest size-class (i.e., earliest colonization stage) on the H14 surface. Although the distribution of the largest size-class on H14 surface does not significantly deviate from CSR, there is a clear peak within the simulation envelope at the same cluster radius as the intermediate and small size class cluster radii (Fig. 4e and Extended data Tables 1,2). This PCF pattern is best explained by the inherent Gaussian nature of the populations resulting in overlap of the first and second generations, so that some of the largest specimens from the second generation are contained within the large size class.

The double Thomas clustering of *Fractofusus* on the ‘E’ and H14 surfaces suggests that these two assemblages represent a similar stage of population development in contrast with the less developed single Thomas clustering seen on the ‘D’ surface. Even so, the taxonomic compositions of the two Mistaken Point communities (the ‘D’ and ‘E’ surfaces) are conspicuously less *Fractofusus* dominated than the effectively monospecific assemblage of *Fractofusus* on the H14 surface (98.4% *Fractofusus*) (SI:2.1). Darroch et al.<sup>7</sup> have further inferred continuous reproduction on the ‘D’ and ‘E’ surfaces based on unimodal size

distributions, whereas the tri-modal size distribution we have documented on H14 points to more intermittent reproduction. Clearly there are significant ecological differences between the two localities, presumably related to background levels of nutrients or disturbance.<sup>61</sup> It is notable, however, that none of these effects obscure the underlying stolon-based reproductive biology of *Fractofusus*

Beyond the three bedding-plane assemblages analyzed here, the next largest documented occurrence of *Fractofusus* is at Bristy Cove, Mistaken Point, which has 76 specimens. Previous nearest neighbour analysis of this assemblage identified a single population of small, randomly distributed specimens (all but one <5.1 cm long).<sup>7,10</sup> Without re-collecting these data (the surface was buried beneath 2 m of storm-surge rubble at the time of our fieldwork and was thus inaccessible), we suggest that this population represents the initial colonization stage in the establishment of a *Fractofusus* community, comparable to the random distribution of largest (colonizing) specimens on H14.

## 2.6 Modes of reproduction

Sessile aquatic organisms have three principal modes of reproduction: waterborne propagules (which includes both spores and submillimetre fragments or buds), larger-scale fragmentation or budding, and via stolon-like runners. Budding and fragmentation are common in corals and sponges, but the existence of complete, sub-centimetre *Fractofusus* at the size we would expect to see either fragments, or morphologically distinct buds, suggests that *Fractofusus* did not reproduce in this way. Furthermore, implied continuous reproduction<sup>7</sup> indicates that buds should always be present, likely in their hundreds based on modern examples in broadly similar depositional environments.<sup>62</sup> Taxonomic groups that reproduce via fragmentation show a high proportion of fragments as gametes.<sup>63</sup> *Fractofusus* and other rangeomorphs found at the sub-centimetre scale are replicas of the adult forms,<sup>14,57,64,65</sup> likely excluding fragmentation as the process behind the analysed spatial patterns. If the fragments were sufficiently small to not be preserved, then their dynamics and therefore spatial distributions would be akin to those of waterborne propagules: it is possible that the first generation resulted from fragmentation, but fragmentation is not considered responsible for the clusters (see main text).

Alternative reproductive processes, such as strobilation and via crawling larvae can also been ruled out, since there is no evidence of gonads, larval trackways, or morphologically different ontological stages for *Fractofusus* across the observed size range.<sup>60,63</sup>

It is not possible to differentiate between sexual and asexual reproduction solely from reproductive mode. While stolon-like reproduction is dominantly asexual, fungal hyphae (such as those in basidiomycete species) are capable of producing fruiting bodies via fusion (anastomosis) of different hyphae mating types (see references within 66). Similarly, waterborne propagation does not equate to sexual reproduction. For example, spores of the

red alga *Polysiphonia* are asexual, and small asexually produced buds or fragments would also show the same distribution patterns as sexually produced gametes. As such, it is not possible to conclusively determine whether *Fractofusus* reproduced asexually or sexually.<sup>19</sup> Even so, there are only a limited number of combinations of reproductive mechanisms that are consistent with the preserved fossil distributions:

- 1) Both sexual and asexual reproduction. Reproduction dominated by asexual stolon interspersed with occasional, sexually produced waterborne propagules.
- 2) Only asexual reproduction. Reproduction primarily via plant/animal like stolon, with sporadic reproduction via (small) waterborne fragments or buds. The relative rarity of *Fractofusus* waterborne propagules found in our model, and the absence of preserved fragments, means that the asexually produced propagules may have been produced sporadically under duress, such as low nutrient levels.
- 3) Only sexual, fungal-like reproduction, where the fruiting bodies are the *Fractofusus* specimens and sexually-produced spores are released.

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