1 **A developmental ontology for the colonial architecture of salps**

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10 **Abstract**

11 Colonial animals are composed of clonal individuals that remain physically connected and

12 physiologically integrated. Salps are urochordates with a dual life cycle including an

13 asexual solitary stage that buds sexual colonies composed of jet-propelling zooids that

14 efficiently swim together as a single unit by multi-jet propulsion. Colonies from different

15 species develop distinct architectures characterized by their zooid arrangement patterns,

16 but this diversity has received little attention. Thus, these architectures have never been

17 formally defined using a framework of variables and axes that would allow comparative

18 analyses. We set out to define an ontology of the salp colony architecture morphospace

19 and describe the developmental pathways that build the different architectures. To inform

20 these definitions, we collected and photographed live specimens of adult and developing

21 colonies through offshore SCUBA diving. Since all salp colonies begin their development

22 as a transversal double chain, we characterized each adult colonial architecture as a

23 series of developmental transitions, such as rotations and translations of zooids, relative

24 to their orientation at this early shared stage. We hypothesize that all adult architectures

25 are either final or intermediate stages within three developmental pathways towards either

26   bipinnate, cluster, or helical forms. This framework will enable comparative studies on

27   the biomechanical implications, ecological functions, evolutionary history, and

28   engineering applications of the diversity of salp colony architectures.

29

30   **Introduction**

31   Salps (Chordata: Tunicata: Thaliacea: Salpida) are marine pelagic urochordates

32   that filter-feed on phytoplankton and bacteria. The salp life cycle (Fig. 1) consists of a

33   solitary stage (oozooid) that asexually buds colonies of the aggregate stage

34   (blastozooids) along a ventral projection (stolon). Aggregate blastozooids are

35   protogynous and can sexually reproduce, brooding embryonic solitary oozooids in a

36   placenta as females (Bone, 1998). While solitary oozooids move using single-jet

37   propulsion (such as solitary medusae), salp aggregate colonies move in an integrated,

38   coordinated manner through multi-jet propulsion.

39   Compared to other multijet colonies, e.g., siphonophores or pyrosomes, salps

40   present a much broader set of architectural configurations among free-swimming

41   colonial animals (Madin, 1990). Salp colony architectures vary across the 48 described

42   species of salps, and include transversal chains, oblique chains, linear chains, whorls,

43   clusters, and helical solenoids (Fig. 2). This diversity and complexity in the

44   arrangements of zooids across species represent a morphological phenotype above the

45   individual's morphology. A familiar analogy would be the variation in the quaternary

46   structure of proteins as an emergent property of lower-level structural changes. While

47   these colonial architectures look radically different from one another, all species have

48   one early developmental stage in common where the stolon of the solitary progenitor

49   segments into a double chain of paired chiral zooids arranged in a transversal double

50   chain (Bone, 1998).

51   As the young colony is released from the solitary, the zooids grow rapidly in size,

52   develop their anatomical features, and in most species, they shift their arrangement in

53   the colony into the different architectures we observe in adults. While these colony

54   architectures have been described qualitatively (Madin, 1990), they have received little

55   attention in the past three decades and lack a formal definition. The primary gaps of

56   knowledge include a breakdown of the traits that define the ontology of these

57   architectures, a quantitative framework to measure those traits, and a detailed

58   comparison of the developmental processes that give rise to the different architectures.

59   An ontology is defined as "a set of concepts and categories in a subject area or domain

60   that shows their properties and the relations between them" (Oxford Languages, 2023).

61   In biology, ontologies serve as conceptual frameworks to designate categories,

62   identities, and relationships of parts and variations in complex systems (Bard & Rhee

63   2004). In this context, we refer to an ontology of salp colony architectures as the

64   categorization of the forms, the description of their characteristics, the definition of their

65   ontogenetic relationships, and their relationships to geometric transformations.

66   Here we aim to leverage the shared earliest stage in their colonial development

67   to (1) define a set of homologous axes, variables, and planes of observation in all salp

68   colonies, (2) map the different architectures based on (1), and (3) define a hierarchical

69   classification of the distinct types and degrees of developmental translations and

70   rotations of the zooids. The ultimate goal is to enable comparative analyses of variation

71   in zooid arrangements between and within architectures. Using this framework,

72   comparative studies will be able to investigate the biomechanical implications,

73   ecological functions, evolutionary history, and engineering applications of the extant

74   architectonic diversity of salp colonies. Moreover, this work will shed light on the

75   broader design space of clonal coloniality among animals.

76

77   **Materials and Methods**

78   We observed and collected live specimens of both adult salp blastozooid

79   colonies and developing colonies in the stolons of solitary salp oozooids. These

80   specimens were collected while SCUBA diving untethered from a small vessel off the

81   coast of Kailua-Kona (Hawai'i Big Island, 19°42'38.7" N 156°06'15.8" W), at an offshore

82   location with a bottom depth of over 2000m. Some dives were conducted during the

83   day, where we encountered most of the specimens of *Iasis cylindrica* (Cuvier, 1804),

84   *Pegea* sp., *Cyclosalpa affinis* (Chamisso, 1819), and *Brooksia rostrata* (Traustedt,

85   1893). The rest of the species included in this study were collected during night dives

86   when many salps perform diel vertical migration to shallower depths. We collected and

87   photographed blastozooids across 22 salp species. In addition, we supplemented our

88   gaps in taxon sampling using underwater photos and videos of live salps from previous

89   expeditions and from online sources. Salps were identified to the species level using

90   stereo microscopy to examine the dorsal tubercules and muscle band distributions,

91   following the taxonomic keys in Yount (1954), van Soest (1974), Godeaux (1998), as

92   well as in Esnal & Daponte (2009). See SM Table 1 for a list of the specimens observed

93   within each species and architectural type.

94   After the dive, salp specimens were anesthetized in 0.2% MS222 buffered with

95   sodium bicarbonate in seawater to facilitate photography. Developing stolons were

96   dissected from the anesthetized solitary oozooid before photographing. We

97   photographed anesthetized adult and developing blastozooid colonies in glass

98   crystallization dishes with a black background using a Canon 6D DSLR camera with a

99   35mm lens mounted on an inverted tripod used as a copy stand. Specimens were

100   photographed from different orientations relative to the constituent zooids' bilateral

101   symmetry (oral, aboral, dorsoventral, and lateral), with a ruler in the frame for scale

102   reference.

103   From these images, we examined the colonial arrangement from the earliest

104   stage of stolon development to adulthood. In some taxa, the temporal axis of

105   blastozooid development can be observed spatially in a continuous gradation of

106   blastozooid development (Fig. 3). This is the case in *Cyclosalpa* spp. (Fig. 3E-F),

107   *Brooksia* spp., *Soestia* spp., and *Helicosalpa* spp. (Fig. 3G). In other taxa, the temporal

108   axis of blastozooid development can also be observed spatially in discretely segmented

109   cohort blocks with synchronous development within each block, such as in *Salpa* spp.,

110   *Ritteriella* spp. (Fig. 3D), and *Thalia* spp. Other taxa, however, produce only a single

111   cohort block with synchronous development, such as in the case of *I. cylindrica* (Fig.

112   3C)*, Thetys vagina* Tilesius, 1802 (Fig. 3B)*, Pegea* spp. (Fig. 3A), and *Traustedtia*

113   *multitentaculata* (Quoy & Gaimard, 1834). We examined the development of the

114   blastozooid chain in these taxa by keeping the solitaries alive in seawater and observing

115   the developmental transitions overnight.

116

Results

Defining the observation framework - The arrangement and relative orientation of

blastozooids in different colony architectures present a 3-dimensional problem, where

the axes and angles of reference shift in ways that are challenging to compare from a

single viewpoint. Using the transversal double-chain architecture found in the earliest

developmental stage of every species (as well as in adult colonies of *Pegea* spp. and

Traustedtia spp.), in addition to the bilateral symmetry of salp blastozooids, we defined

three orthogonal axes and their corresponding normal planes (Fig. 4). These are: (1)

The dorsoventral axis of the colony is defined as the axis parallel to the dorsoventral

axis of the zooids in the transversal double chain, with a normal (perpendicular) plane of

observation corresponding to viewing the dorsal side of the zooids on either side of the

transversal double chain. (2) The oral-aboral axis is defined as the axis parallel to the

oral-aboral axis of the zooids in the transversal double chain, with a perpendicular plane

of observation corresponding to viewing the oral or aboral end of the zooids on either

the frontal or rear side of the transversal double chain. The zooid oral-aboral axis of

each zooid is defined as the line parallel to the endostyle. (3) The stolon axis is defined

as the axis of chain growth parallel to the stolon, perpendicular to a plane of observation

that corresponds with looking directly at either end of the transversal double chain, with

a lateral view of the zooids.

With these axes and planes delineated, we can then describe developmental

changes in zooid orientation characters relative to these three planes of observation.

Based on this universal observation framework, we then defined the following

characters: (A) the dorsoventral zooid-stolon angle is the angle formed between the

140   oral-aboral axis of the zooid and the elongation axis on the colony as viewed from the

141   dorsal side of the developing zooids, driving the formation of oblique, linear, and

142   bipinnate chains; (B) the lateral chiral angle, defined as the angle formed between the

143   oral-aboral axes of a pair of chiral zooids as viewed from the zooids' lateral orientation,

144   driving the formation of bipinnate chains; (C) zooid autorotation, defined as rolling of the

145   zooid around its own oral-aboral axis also driving the formation of bipinnate chains; (D)

146   serial stolon-normal angle, defined as the angle formed between a zooid's oral-aboral

147   axis and the oral-aboral axis of its lateral neighbor as viewed from one end of the

148   colony, driving the formation of a solenoid double helix chains; (E) peduncle length ratio

149   (the peduncle is an extension of the tunic that connects the zooids to their chiral pair or

150   to the stolon during development, present in most *Cyclosalpa* species), defined as the

151   ratio between the total oral-aboral length of the zooids relative to the longest axis of

152   their peduncle, driving the formation of whorls and clusters; and finally (F) neighbor

153   attachment, defined as direct contact between lateral neighbors, its loss drives the

154   formation of clusters with loosely-attached zooids.

155   We examined changes in these variables across the development of colonies in

156   different salp species and characterized a developmental ontology of salp colony

157   architecture by first describing the set of developmental transformations that give rise to

158   each architecture, then identifying which intermediate stages in the formation of more

159   derived architectures in some species are equivalent to the adult finalized architectures

160   in other species, to build a process-based hierarchical ontology of the architectures

161   within colonial developmental pathways.

162   ***A developmental ontology of architectural transition pathways –*** In congruence

163   with Madin (1990), we observed that the earliest stages in the development of salp

164   colonies across all species display a transversal double chain architecture which

165   undergoes subsequent changes towards the adult colonial architecture.

166   In some taxa, such as *Pegea* spp. (with adult and developing forms examined in

167   this study) and *Traustedtia* spp. (observed in photographs from other divers), we

168   noticed that the transversal double-chain architecture remains unchanged throughout

169   the growth and development of the blastozooids in the chain. This architecture is

170   characterized by a dorsoventral zooid-stolon angle of ~90°, with ventral attachment to

171   the chiral neighbor and lateral attachment to the lateral neighbors (Fig. 2A). In the field,

172   we observed these chains moving parallel to the oral-aboral axis of their zooids, at an

173   angle orthogonal to the length of the chain. Often, we find colonies of *Pegea* species

174   moving in a coiled formation, where the transversal chain is curled up on the oral-

175   aboral-normal plane. Other species we examined do not retain this developmentally

176   basal architecture, but instead modify the orientation, rotation, and position of the zooids

177   relative to each other and the axis of the chain during development (Fig. 5). Our

178   observations on the developmental series of salp species revealed intermediate stages

179   between the initial transversal arrangement and the final adult arrangement (terminal

180   architecture). In the case of the most elaborate terminal architectures (such as bipinnate

181   chains, linear chains, and clusters) their intermediate stages shared the diagnostic

182   characteristics of other simpler terminal architectures. We summarized the

183   developmental relationships between architectures as three hypothesized

184 developmental transition pathways (depicted in Figure 6), each characterized by

185 specific zooid rearrangement processes.

186   First there is the hypothesized pathway that leads to the architectures found in

187 *Cyclosalpa* spp. These blastozooid chains continue their development in a transversal

188 arrangement (dorsoventral zooid-stolon angle of 90°) but grow peduncles that separate

189 the zooids from the stolon attachment point and from their chiral ventral neighbor.

190 Moreover, discrete sets of budding salps bundle together, where the attachment points

191 of their peduncles remain attached to a central point and detached from other sets.

192 These radial aggregations (whorls) are formed by two chiral, bilaterally symmetrical,

193 semicircular sets of zooids (Ritter & Johnson 1911). In the first stage of the

194 transformation, we observe the formation of the whorl architecture (e.g., Fig. 3E). The

195 zooids in this architecture are packed together tightly in a wheel shape due to the short

196 peduncles (Fig. 2B). We found this terminal architecture and observed its development

197 in some *Cyclosalpa* species such as *C. affinis, C. quadriluminis* Berner, 1955*,* and *C.*

198 *bakeri* Ritter, 1905. In *C. affinis*, we saw these whorls remaining attached to each other

199 for a longer time than in other species and adult whorls can often be found conjoined.

200 We hypothesize that a further (more developmentally derived) stage in this

201 transformation is the cluster architecture, where after reaching the 'whorl' intermediate

202 stage, the peduncles grow longer to the point that zooids are no longer attached

203 laterally to each other (Fig. 2C) and can freely bob around and end up arranged in

204 hemispherical or spherical sets. We found the cluster terminal architecture in other

205 *Cyclosalpa* species such as *C. sewelli* Metcalf, 1927*, C. pinnata* (Forskål, 1775)

206 (observed in the field but not photographed for this study)*,* and *C. polae* Sigl, 1912*.* We

207   observed the development of cluster architectures from a transversal stage in *C. polae*

208   and *C. sewelli*. These cluster bundles appeared to contain many more zooids than

209   those in whorls. From our observations of the development of *C. sewelli*, we were not

210   able to discern whether they ever go through a distinct 'whorl' stage the way that *C.*

211   *polae* definitively does. The developing cluster and whorl colonies we observed all went

212   through a similar zooid-stolon allometry, though we were unable to determine whether

213   the serial neighbor zooid detachment (Fig. 6) occurs before or after the release of the

214   radial aggregations.

215   Second, there is the hypothesized pathway leading to the helical double-chain

216   architecture in *Helicosalpa* spp. (Fig. 2D). While we did collect and examine adult

217   colonies of *H. virgula* and *H. younti*, we did not encounter any *Helicosalpa* oozooids with

218   developing colonies in which to observe the developmental transition. However, we

219   could observe their development in a photograph by David Wrobel (Fig. 3G), and in

220   Figure 2 of Ringvold et al. (2020). Like the other salps we observed, these double

221   chains are budded in a transversal arrangement, but subsequently undergo stolon

222   torsion into a solenoid shape (Fig. 3G), where zooids become angled relative to their

223   chiral pair to accommodate this conformation. Then there is the hypothesized pathway

224   leading to streamlined linear chains through the alignment of zooid orientations to the

225   axis of the stolon during development. We observed the development of linear chains

226   from transversal stages in *Iasis cylindrica* and noticed they start with a partial

227   dorsoventral rotation of zooids at an angle within the range of oblique terminal

228   architectures (Fig. 3B), which is the final form for species like *Thetys vagina* (as

229   mentioned in Madin 1990) and *Thalia* spp. (at least in *T.longicauda* and *T. cicar*

230　examined in this study, as well as in *T. democratica* observed in other sites by the

231　authors, see example in Fig. 2E), with zooid-stolon angles between 50-60°. Therefore,

232　we hypothesize that the swimming direction of these oblique colonies is closely aligned

233　(but not perfectly parallel) with the stolon axis. In subsequent stages of the development

234　of linear colonies, we observed this dorsoventral torsion going further toward near-

235　complete alignment (15-30°) of the oral-aboral axis of the zooids to the axis of the

236　stolon. We observed a terminal linear architecture in *Ihlea punctata, Iasis cylindrica,*

237　*Metcalfina hexagona*, *Soestia zonaria* (Pallas, 1774), and several *Salpa* species. In the

238　species *S. zonaria*, we find the most extreme version of this architecture, with zooid-

239　stolon angles close to 0° (Fig. 2F). Finally, based on similarities between the zooid

240　orientations between bipinnate and linear terminal architectures, we hypothesize that

241　the bipinnate architecture (which we observed in *Brooksia rostrata, Ritteriella*

242　*amboinensis,* and *Ritteriella retracta*) represents a derived transformation stage in the

243　linear pathway. We observed that both linear and bipinnate architectures share the

244　linear dorsoventral alignment of zooids to the stolon. However, adult colonies in the

245　species we observed having the bipinnate architecture additionally displayed a

246　symmetrical outward lateral flare of the aboral ends of zooids in the oral-aboral-normal

247　plane and an autorotation of zooids where the ventral (and dorsal) sides of every zooid

248　are all facing the same side of the colony (Fig. 2G). The developmental transitions in

249　bipinnate species was challenging to observe empirically because *Ritteriella* does not

250　undergo transformation past the oblique stage in colonies retained by the oozooid (Fig.

251　3D), and in *Brooksia* the transformation occurs at a very small scale in the most

252　proximal and underdeveloped end of the budding colony. It is possible that the order of

11

253   developmental transitions that lead to the bipinnate morphology differs from the one

254   hypothesized in Figure 6. However, our observations on developing *Ritteriella* colonies

255   indicate that these transformations occur during or after the process of dorsoventral

256   zooid stolon rotation that produces the oblique intermediate form.

257   Each developmental transition is characterized by variation across specific

258   continuous morphological traits (Fig. 6), which we describe based on the differences in

259   zooid orientation and shape between the initial budding transversal chain stage and the

260   final adult stage supplemented by some observations of the developmental changes.

261   We hypothesize that the transversal-to-whorl transformation is mediated by an increase

262   in the peduncle-to-zooid length ratio and a continuous allometric shift in zooid-to-stolon

263   size as the zooids grow and develop asynchronously along the stolon length. The

264   subsequent whorl-to-cluster transformation also seems to rely on further peduncle

265   elongation but is marked by a loss of neighbor zooid attachment that allows neighboring

266   zooids to bob around freely. The transversal-to-helical pathway (based on observations

267   made on the photograph in Fig. 3G) is characterized by a continuous shift in the serial

268   neighbor stolon-normal angle, where the orientation of neighboring zooids breaks

269   parallelism and starts to offset by a few degrees like stairs in a spiral staircase. The

270   transversal-to-oblique-to-linear pathway appears to be driven solely by changes in the

271   dorsoventral zooid-stolon angle based on our observations. Finally, we hypothesize that

272   the linear-to-bipinnate transformation is driven by an increased oral-aboral chiral angle

273   and zooid autorotation, where the oral-lateral facets of chiral zooids face each other, the

274   aboral ends turn outwards, and their ventral sides face the same side of the colony.

275   However, we were not able to observe the development of bipinnate colonies past an

276   oblique stage, so the order in which these modifications occurred remains unknown.

277

278   **Discussion**

279   Ontologies in biology are helpful conceptual tools to characterize, categorize, and

280   compare variation between and within species. We leveraged similarities in the

281   development of salp colonies across species to categorize and geometrically compare

282   the different architectures. From this developmental perspective, we were able to

283   propose a hypothesis-generating ontology for salp colony architecture describing the

284   developmental transitions in the zooid arrangements that lead to the different terminal

285   architectures. Through this ontology, we hypothesize that some terminal architectures

286   are homologous to intermediate stages in the development of other terminal

287   architectures. These ontological definitions and reference frameworks are essential to

288   measure and compare standing variation in colony architecture and its emergent

289   properties between salp species.

290   One of the most immediate emergent properties of salp colony architecture is the

291   potential implications for locomotion. Different salp colony architectures present different

292   relative orientations of the individual jets to each other and to the overall colony motion

293   axis. In addition, we hypothesize that different architectures differ in how the number of

294   zooids in the colony scales with cross-sectional area relative to motion. These

295   hydrodynamic properties can have further consequences on the locomotory efficiency of

296   different architectures. Swimming in linear salp chains is hypothesized to be more

297   economical due to the reduction of drag (Bone & Trueman 1983). A salp colony is

298   equipped with multiple propelling jets rather than one, which increases its propulsive

299   power. Drag experienced during swimming depends on the total area exposed to the

300   fluid as well as the frontal (motion-orthogonal) projected area (Alexander 1968). Skin

301   drag will increase with the number of zooids in the colony in a predictable manner that is

302   independent of their zooid arrangement. However, frontal drag is drastically reduced in

303   linear chains compared to the sum of each separate zooid (Mackie 1986). We

304   hypothesize that frontal drag will vary across architectures and therefore impact the

305   relative speed attained by each species. In addition to changing the way the frontal area

306   scales with the number of zooids, we hypothesize that architecture may also impact the

307   angles of the jets relative to the axis of colony motion. In siphonophores, the velum of

308   the nectophore is used to orient the jet to prioritize torque or thrust (Sutherland et al

309   2019). In salps, these orientations are usually fixed in a colony (Sutherland & Weihs

310   2017), but the angle of the exhalant jets relative to the swimming of the colony will

311   dictate the thrust-to-torque ratio, which will determine their propulsive efficiency.

312   Understanding the hydrodynamic advantages and implications of each colonial

313   architecture can be valuable beyond basic science since it may yield interesting

314   applications to bioinspired underwater vehicles. Pulsatile jet propulsion is increasingly

315   inspiring underwater vehicle engineering (Mohensi 2006, Yue et al. 2015). Multijet

316   systems comprised of collaboratively interactive propeller units could revolutionize the

317   field of underwater vehicles (Chao et al. 2017, Costello et al. 2015) with designs

318   inspired by gelatinous invertebrates such as salps (Marut 2014, Krummel 2019, Bi et al

319   2022). Some of these bio-inspired solutions are stimulating novel solutions in the field of

320   soft robotics (Renda et al. 2015, Krummel 2019), as deformable body shapes can

321   augment propulsive forces (Giorgio-Serchi & Weymouth 2017). Understanding the

322   biomechanical underpinnings of the diversity of salp colony architectures would reveal

323   nature's broadest design space for underwater multi-jet-propelled soft locomotors and

324   their inherent trade-offs.

325   Another potential contribution of this architectural ontology is the characterization

326   of colonial morphology from a comparative, evolutionary perspective. Salp colony

327   architectures are distributed across the phylogenetic diversity of salp species, but their

328   evolutionary history remains unknown. The two main obstacles to the reconstruction of

329   the evolutionary history of salp colony architecture have been (1) the lack of a

330   framework to compare and characterize variation between architectures, and (2) a

331   phylogenetic tree that resolves the position of every architecture in every lineage where

332   it has evolved. Metcalf (1918) hypothesized phylogenetic relationships among salps

333   based on gut morphology, with *Cyclosalpa* as the most distant relative to other salps

334   due to its linear gut shape. Half a century later, Madin (1974) hypothesized that colonial

335   architecture is phylogenetically conserved and that fast-swimming linear and bipinnate

336   chains are monophyletic. Years later, Tsakogeorga et al. (2009) reconstructed the first

337   molecular phylogeny using 18S sequences to resolve relationships between thaliacean

338   groups, supporting the monophyly of salps. Following this work, Govindarajan et al.

339   (2011) included a more extensive taxon sampling within salps, revealing that salps with

340   linear chain architectures are not monophyletic, and that the transversal architecture in

341   *Pegea* is likely derived, despite having been hypothesized as ancestral (Madin 1990).

342   While this phylogeny included many of the known salp species, it cannot fully resolve

343   the evolutionary history of salp colony architecture since the position of *Pegea* and

344   *Thalia* are poorly resolved and the position of *Helicosalpa* is unknown. A phylogenetic

345   comparative approach to the diversity of colonial architectures will facilitate further

346   research on its evolutionary, ecological, and biomechanical underpinnings. If

347   evolutionary shifts in the architecture of salp chains bring on changes in their

348   locomotory efficiency, it is possible that these shifts are related to different selective

349   pressures such as predatory pressure, habitat nutritional patchiness, or vertical

350   migration behavior.

351   The research directions outlined above would advance our understanding of salp

352   biology across their species diversity. Salps have attracted significant scientific interest

353   in the past decade since they are essential consumers in oceanic ecosystems that feed

354   on microbial plankton production and can grow explosively following phytoplankton

355   blooms (Henschke et al. 2016). Salp fecal pellets play an important role in the biological

356   carbon pump and are responsible for a large fraction of the biological carbon pump

357   (Decima et al. 2023), responsible for the trapping of gigatons of carbon fixed from the

358   atmosphere into the deep sea and are therefore a key mediator for atmospheric CO_2

359   concentration and global change (Buesseler et al. 2020). Many of the salp species that

360   contribute most to this process are vertical migrators that respire and deposit (by

361   defecation and predation) carbon during the day in the mesopelagic zone, after feeding

362   during the night near the surface (Steinberg et al 2023). Many of the vertically migrating

363   salp species (such as *Salpa* spp.) present a linear architecture (Madin et al. 1996) and

364   their migratory behavior varies with colonial development (Henschke et al. 2021). While

365   some of these linear, vertically migrating species have been extensively investigated,

366   the ecology and natural history of the broader diversity of salps remains understudied.

367   Characterizing the relationship between colonial architecture, locomotion, and migratory

368   behavior is key to understanding the ecological implications of shifting salp species

369   compositions and distributions with global change (Lavaniegos & Ohman 2003).

370   Finally, we believe the colonial ontology proposed here expands our

371   understanding of the development and evolution of colonial animals in general. Colonial

372   animals (modular colonies, not including eusocial colonies) are composed of clonal

373   individuals produced by asexual reproduction that remain physically connected and

374   physiologically integrated (Harvell, 1991). Most animal colonies are arranged with their

375   zooids in parallel to each other forming 2D sheets with one pole, typically the oral end,

376   exposed to the external environment. Some of these topologically simple planar

377   colonies can form complex 3D shapes by folding this sheet. In benthic species, the

378   sheet is often folded around an endogenous skeleton or an object in the environment

379   (e.g., corals, ascidians, millepores). Bryozoans also tend to develop into sheets though

380   also into branching structures. In pelagic species, colonies are free-living and capable of

381   swimming around by the combined (and often coordinated) action of their zooids (Du

382   Clos et al. 2022). Therefore, their shapes are often directional, with a front and a rear

383   end defined by their colonial locomotion (Mackie, 1986). On one hand, pyrosomes

384   (Chordata: Tunicata) use the same 2D-sheet template as their benthic relatives, yet in

385   their case, the sheet grows folded forming a closed-ended tube where all the exhalant

386   flow from the inner side aboral ends of the zooids are canalized to a single jet stream.

387   On the other hand, siphonophores and doliolids typically form 1D colonies with sub-

388   specialized zooid types, where only one or few frontal locomotory zooids (nectophores

389   and nurse zooid, respectively) propel a linear colony with non-swimming zooids

390   dragging behind. Siphonophore (Cnidaria: Hydrozoa) colonies can be topologically

391   complex in benthic rhodaliids or in the pleustonic Portuguese Man-o-war, but most

392   planktonic free-swimming siphonophore colonies have their zooids arranged bi-serially

393   or mono-serially along a stem (Mackie et al., 1988). Among them, physonect

394   siphonophores bear multiple nectophores (swimming bodies) that propel the colony

395   through multijet propulsion (Sutherland et al. 2019) in a similar fashion to linear salp

396   colonies. Compared to siphonophores or pyrosomes, salps present a much broader set

397   of architectural configurations among free-swimming colonial animals (Madin, 1990),

398   thus expanding the boundaries of our known design space for both form and function of

399   coloniality in the pelagic realm.

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512

513   **Tables**

514

515   **Figure Legends**
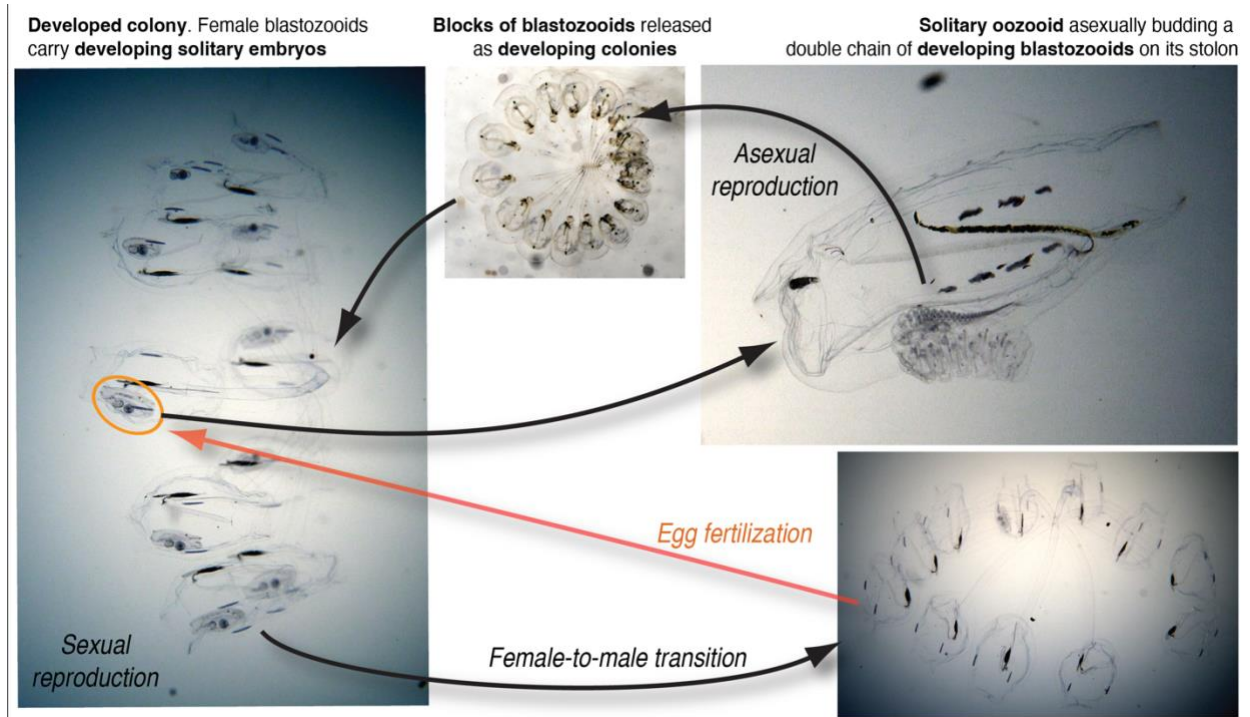
Figure 1. Salp life cycle using the species *Cyclosalpa sewelli* as an example. Frame captures from brightfield in situ videos by Brad Gemmell following method from Colin et al. 2022.

520
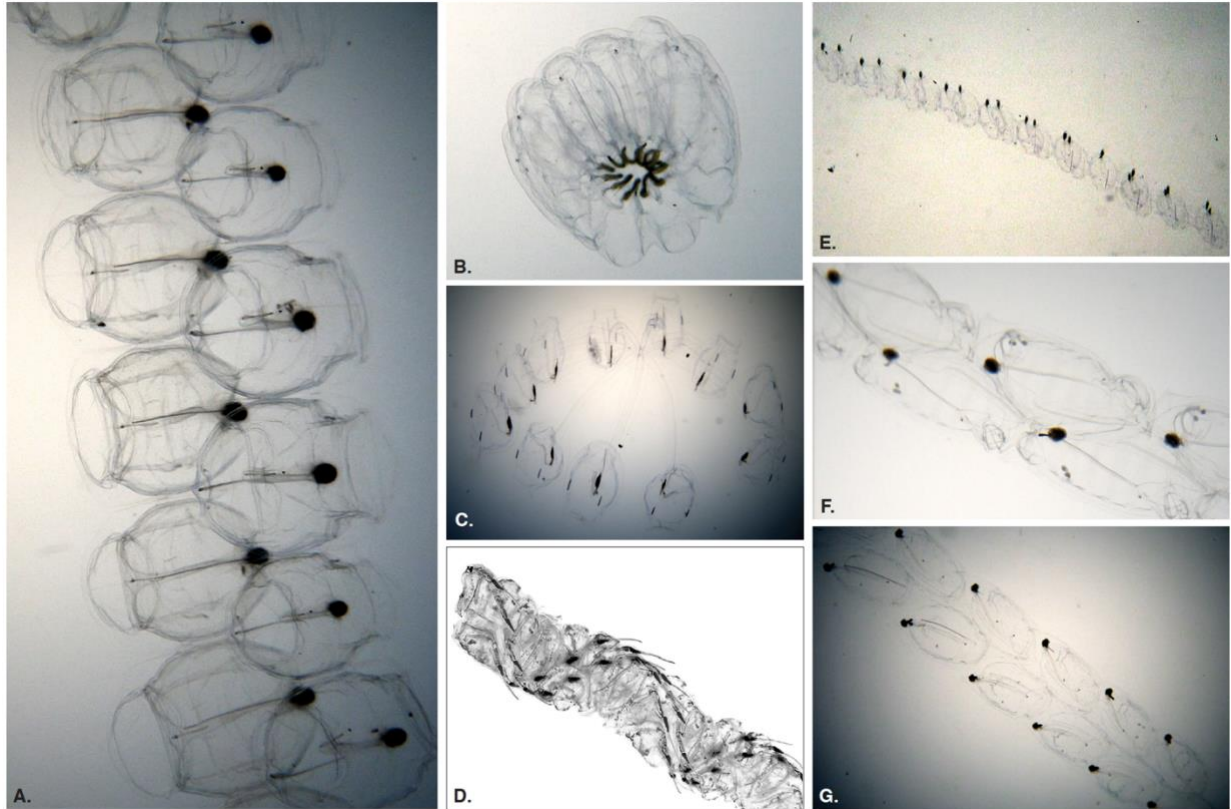
Figure 2. Adult salp colonies representing every distinct colonial architecture observed
across all salp species. (A) transversal chain (*Pegea* sp.), (B) whorl (*Cyclosalpa affinis*),
(C) cluster (*Cyclosalpa sewelli*), (D) helical chain (*Helicosalpa virgula* (Vogt, 1854),
photograph by Nils Aukan), © oblique chain (*Thalia longicauda* (Quoy & Gaimard,
1824)), (F) bipinnate chain (*Ritteriella amboinensis* (Apstein, 1904)). Frame captures A,
B, C, E, F, G from brightfield in situ videos by Brad Gemmell.
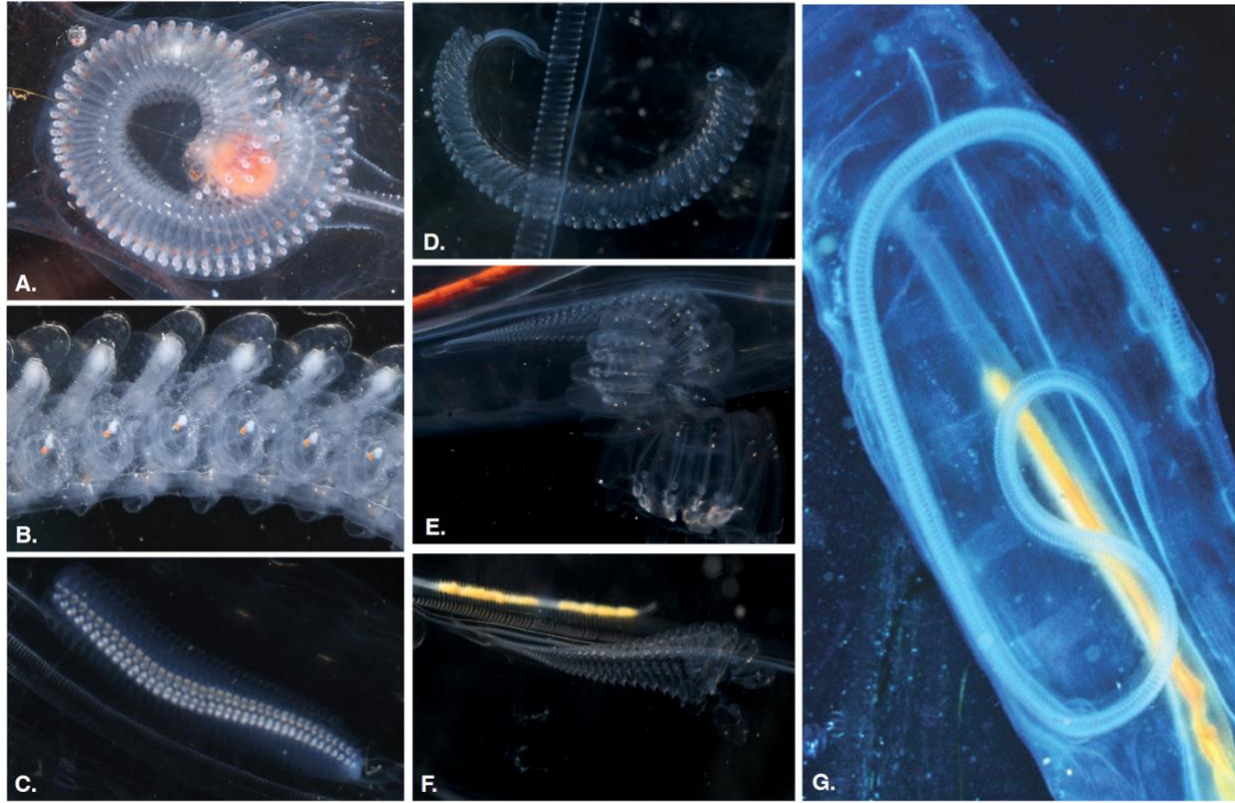
Figure 3. Developing blastozooid colonies produced by the budding stolons of solitary oozooids across different salp architectures. (A) transversal chain buds (*Pegea* sp.), (B) oblique chain buds (*Thetys vagina*), (C) linear chain buds (*Iasis cylindrica*), (D) bipinnate chain buds (*R. amboinensis*), (E) whorl buds (*C. affinis*), (F) cluster buds (*Cyclosalpa polae*), (G) helical chain buds (*H. virgula*, photograph by David Wrobel).

A.

B.

Dorsoventral ———   Oral-Aboral ———   Stolon ———

C.

533
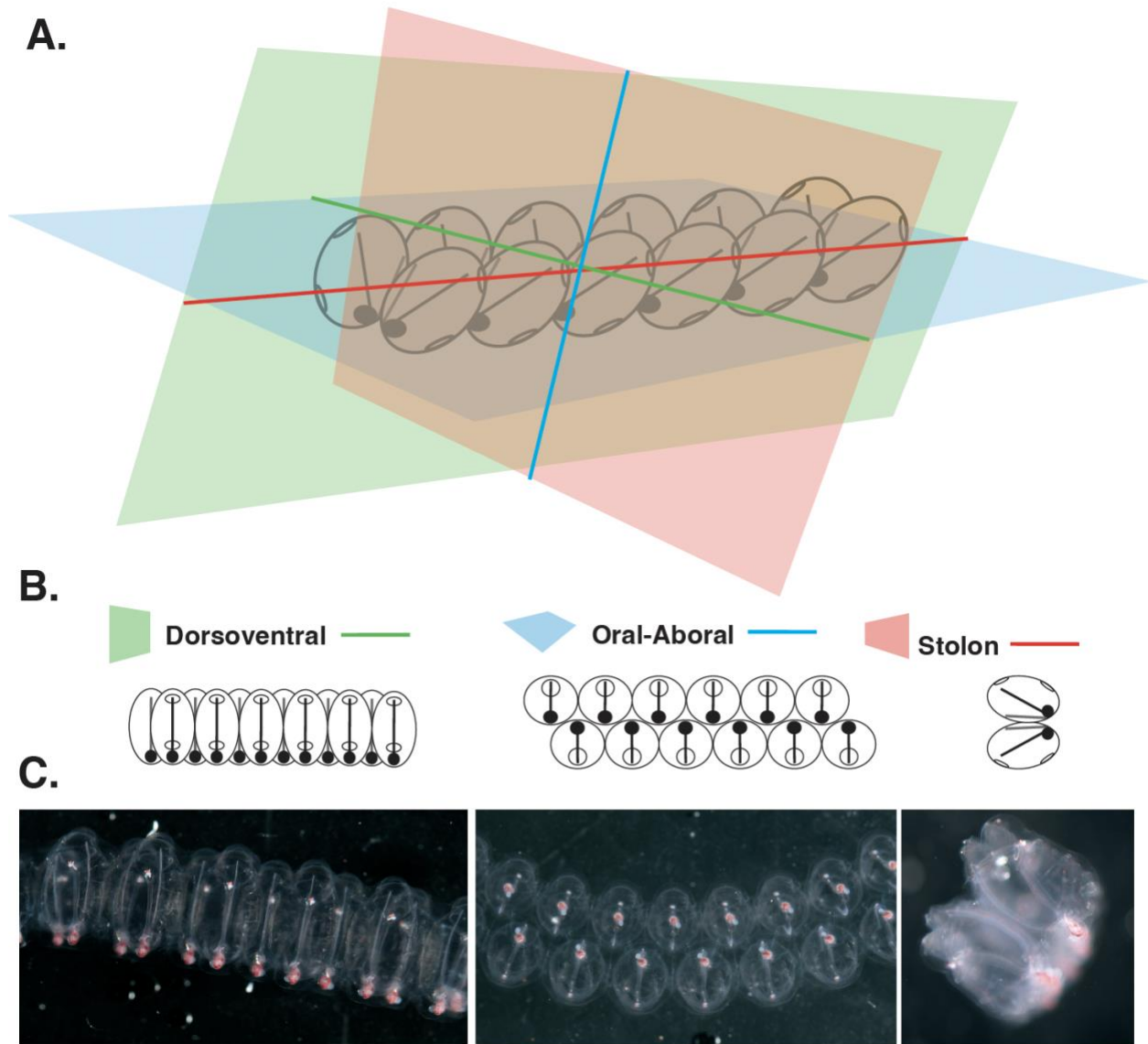
534   Figure 4. (A) Definition of the homologous universal axes and planes of observation

535   relative to the orientation of the colony in the early transversal chain stage. (B)

536   Diagrams representing the cross-sectional views of a transversal chain from each of

537   these three planes of observation. (C) Photographs of a transversal chain of early-

538   developing *Pegea* sp. blastozooids taken from each of the abovementioned

539   orientations.

540

541

Figure 5. Sketches of individual zooids and adult colonies representative of every

architecture as viewed from each plane of observation, with key structures labeled. The

rightmost column lists the species in which these terminal architectures were observed.
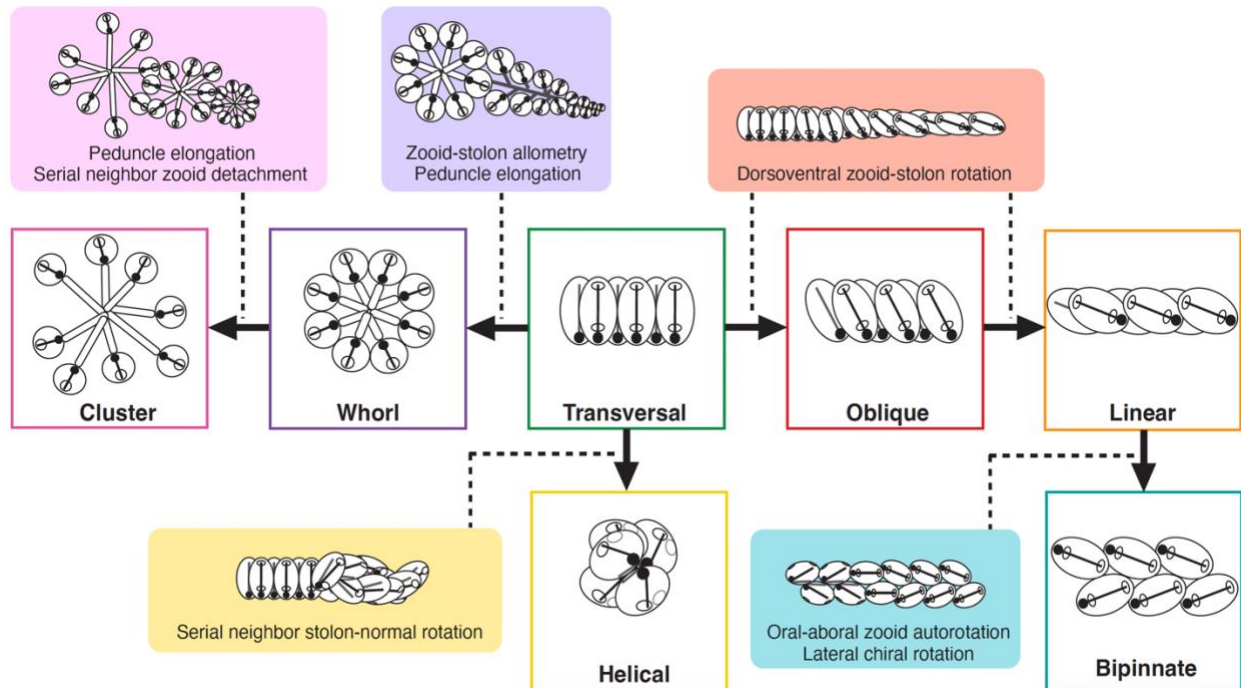
Figure 6. Hypothesized developmental transitions (solid arrows) and mechanisms involved (solid color boxes associated with each transition by a dotted line) leading to the different adult blastozooid colony architectures (white boxes with colored outlines). All developmental mechanisms are based on observations of colonial development, except those involved in the bipinnate architecture, which are hypothetical and based on comparisons between adult architectures. The transversal architecture is found in the earliest developmental stage of every species as well as in the adult stage of some species and has been hypothesized to be ancestral to all salps (Madin 1990).

Ethical Care Considerations

Our specimen collection and protocol were compliant with all local regulations. Since no vertebrates or cephalopods were involved, we did not need oversight from an animal care board.

560 **Data Accessibility**

561 All photographs will be made available in a Dryad repository.

562

563 **Supplementary Data**

564 SM Table 1. Number of images examined from salp specimens of each life cycle stage

565 and species. Specimens collected visa SCUBA diving off Kona are shown as numbers

566 in black. Other images from different sources are highlighted in red.

Species	Architecture	Adult colony specimens	Total adult colony specimens	Developing colony specimens	Total developing colony specimens
Pegea sp.	Transversal	5	10	4	5
Pegea confoederata	Transversal	4 (Semenov, LJL videos)			
Pegea socia	Transversal	2 (R/V Sikuliaq 2022)			
Pegea bicaudata	Transversal	1 (Ryo Minemizu)			
Traustedtia multitentaculata	Transversal	1 (Dennis Rabeling)		1 (Suzan Meldonian)	
Cyclosalpa affinis	Whorl	3	10	1	6
Cyclosalpa bakeri	Whorl	4		2	
Cyclosalpa quadriluminis	Whorl	3		3	
Cyclosalpa polae	Cluster	5	9	4	7
Cyclosalpa sewelli	Cluster	3		3	
Cyclosalpa pinnata	Cluster	1 (Kona GoPro footage)			
Helicosalpa younti	Helical	1	2		2
Helicosalpa virgula	Helical	1		2 (D. Wrobel , Ringvold et al. 2020 Fig. 2EF)	
Helicosalpa komaii	Helical	1 (Ringvold et al. 2020 Fig. 4)			
Thalia cicar	Oblique	1	6	1 (KAUST formalin specimen)	2
Thalia longicauda	Oblique	2			
Thalia democratica	Oblique	1 (David Shale)			
Thetys vagina	Oblique	2 (NaturePL, MBA)		1	
Iasis cylindrica	Linear	6	18	6	14
Ihlea punctata	Linear	1		1	
Metcalfina hexagona	Linear	1			
Salpa aspera	Linear	1			
Salpa fusiformis	Linear	2		4	
Salpa maxima	Linear	3			
Salpa thompsoni	Linear	1 (LJL video)		1 (Mike Stukel)	
Soestia zonaria	Linear	3		2 (FSU photos)	
Brooksia rostrata	Bipinnate	3	9	2	6
Rittereilla amboinensis	Bipinnate	3		3	
Ritteriella retracta	Bipinnate	3		1	

567