A stage and anatomy ontology for echinoderm embryogenesis

Charles A. Ettensohn1, Macie M. Chess1, Laurent Formery3#, Troy J. Pells2, Peter D. Vize2, Jenifer C. Croce3

1Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, USA

2Department of Biological Sciences, University of Calgary, Calgary, AB T2N 1N4, Canada

3 Sorbonne Université, CNRS, Institut de la Mer de Villefranche (IMEV), Laboratoire de Biologie du Développement de Villefranche-sur-Mer (LBDV), Evolution of Intercellular Signaling in Development (EvoInSiDe), Villefranche-sur-Mer, France

# Current address:

Department of Biology, Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA.

Author for correspondence:

Dr. Jenifer C. Croce

Institut de la Mer de Villefranche

Laboratoire de Biologie du Développement de Villefranche-sur-Mer

181 Chemin du Lazaret

06230 Villefranche-sur-Mer France

Email: [jenifer.croce@imev-mer.fr](mailto:jenifer.croce@imev-mer.fr)

Tel: +334 93763799

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**Abstract**

Developmental and anatomical ontologies, which consist of developmental staging and anatomical feature series, are fundamental tools in biological research. They enable the standardization of experimental data, thereby enhancing the reproducibility of experiments and facilitating the comparison and integration of information across laboratories and animal species. Ontologies are also essential for the digital curation, dissemination, and analysis of diverse types of biological data, including gene expression patterns and phenotypes associated with pharmacological, molecular, and genetic perturbations. For these reasons, ontologies are considered integral components of model organism knowledgebases. Sea urchins and other echinoderms have been prominent experimental models in developmental biology for more than a century. They are also widely used to explore questions at the interface between evolution and development, due to their phylogenetic position as invertebrate deuterostomes and the striking diversity of developmental programs within the phylum. However, no formalized ontology has been produced this far for any echinoderm. Here, we provide developmental and anatomical ontologies for five echinoderm species, which together represent euechinoids, cidaroids, and sea stars. Moreover, we leverage these ontologies to create a "pan-echinoderm" developmental and anatomical ontology, which will support comparative analyses across the phylum. These resources will be critically important for the digital curation of experimental data from multiple species by Echinobase, the public knowledgebase of echinoderm genomics and development, and by Marimba, the public knowledgebase of invertebrate marine models. More importantly, they will greatly facilitate the use and enhance the value of echinoderms as model organisms for biological research.

**Introduction**

For more than a century, sea urchins, sea stars, and other echinoderms have been prominent experimental models for the analysis of developmental processes (Davidson, 1986; Ettensohn et al., 2004; Ernst, 2011; McClay, 2011). Their popularity, as biological models, stems from their external mode of development and rapid embryogenesis, the ease with which large numbers of gametes and of synchronously developing embryos can be obtained, and the optical transparency and anatomical simplicity of their embryos. Over the years, thousands of published studies have examined the embryonic development of sea urchins, sea stars, and other echinoderms, with a strong emphasis on species that exhibit indirect development; i.e., species with a feeding larva (Wray, 2022). These studies have provided fundamental insights regarding oocyte polarity, fertilization, gene activity in early development, cell fate specification, morphogenesis, and embryonic patterning (Ettensohn and Sweet, 2000; Angerer and Angerer, 2003; Ernst, 2011; McClay, 2016). Recently, the application of powerful molecular and genomics-based tools has made echinoderms, especially sea urchins and sea stars, leading models for the analysis of developmental gene regulatory networks (GRNs), including studies of GRN architecture, function, and evolution (Martik et al., 2016; Peter and Davidson, 2016; Cary and Hinman, 2017; Lowe et al., 2017; Shashikant et al., 2018).

When considering all echinoderm clades, i.e., sea urchins (echinoids), sea stars (asteroids), sea cucumbers (holothuroids), ophiuroids, and crinoids (Telford et al, 2014), species currently used by the research community span a wide range of divergence times, ranging from a few million years to almost half a billion years. There are examples of developmental processes that have been conserved across the longest of these time scales as well as striking examples of developmental novelties that have evolved more recently (Hinman and Davidson, 2007; Koga et al., 2014; Thompson et al., 2015; Morino et al., 2016; Wessel, 2016; Semmens and Elphick, 2017; Dylus et al., 2018; Hinman and Burke, 2018; Gildor et al., 2019; Valencia et al., 2021; Yamazaki et al., 2021; Czarkwiani et al., 2022; Davidson et al., 2022; Khor and Ettensohn, 2020; Khor and Ettensohn, 2022; Levin et al., 2022; Paganos et al., 2022; Wray, 2022). The recent emphasis on comparative developmental studies using echinoderms has been supported by a rapidly expanding collection of genomic datasets of all kinds, including chromosome-level genome assemblies from many echinoderm species (Davidson et al., 2020; Warner et al., 2021; Marlétaz et al., 2023; Galià-Camps et al., 2024; Han et al., 2024; Parey et al., 2024; Polinksi et al., 2024; Zhang et al., 2024; Zhong et al., 2024). As such, there is thus an urgent need to develop tools that facilitate comparison and integration of experimental data across diverse echinoderm clades.

Ontologies are essential tools in biological research (Bard and Rhee, 2004; Strömbäck et al., 2007). They serve to standardize nomenclature, providing a controlled vocabulary for each entity they include. They further define parental, anatomical, and developmental relationships among the entities. As such, they are key to provide a thorough, structured, and formal description of a developing organ or animal (e.g., Richter et al., 2010; van Slyke et al., 2014), making information more accessible. Among ontologies, developmental ontologies correspond to developmental staging series that standardize developmental phases, periods, and stages in a manner independent of external variables such as temperature, culture medium, or embryo batch. They describe and categorize the sequential steps of an organism development in a chronological way based on observable morphological or structural changes. Anatomical ontologies consist of a “parts list” of the developing organism, which includes cell types, germ layers, cell lineages, extracellular layers, tissues, organs, and other anatomical entities, organized and classified in a formal, computationally accessible manner. As a consequence, ontologies are key to standardize experimental data, enhancing their reproducibility and making possible the integration of information across laboratories and model species. They are further critically important for the digital curation and dissemination of biological data, including gene expression patterns and phenotypes associated with control conditions as well as pharmacological, molecular, and genetic perturbations.

Given the important features of ontologies, it is not surprising that they are now considered integral components of most major model organism knowledgebases (Segerdell et al., 2008; van Slyke et al., 2014). Over the past decades, several developmental staging and anatomical series have been constructed for almost all major developmental models. More than 60 years ago, for instance, the growing utility of the chick embryo led Hamburger and Hamilton (1951) to produce a 46-stage series, which is still used today. Developmental ontologies have also long been available for most other major experimental models, including mouse (Theiler, 1972; Kaufman, 1992), zebrafish (Kimmel et al., 1995), and frog (Nieuwkoop and Faber, 1994). More recently, anatomical ontologies for these model organisms have also been produced, i.e., for mouse (Hayamizu et al., 2013), zebrafish (Belmamoune and Verbeek, 2007), and frog (Segerdell et al., 2008; 2013). It is also recently that developmental staging and anatomical series have emerged for the ascidian *Ciona intestinalis* (Hotta et al., 2020) and the lancelet *Branchiostoma lanceolatum* (Bertrand et al., 2021; Carvalho et al., 2021). Surprisingly, despite the rich history and continued prominence of sea urchins, sea stars, and other echinoderms as developmental model organisms, no formalized ontologies for embryonic development have been published for any echinoderm, even though thorough developmental descriptions are available for three echinoids, *Paracentrotus lividus* (Formery et al., 2022), *Lytechinus pictus* (Nesbit and Hamdoun, 2020), and *Lytechinus variegatus* (Morill and Marcus, 2005).

To overcome this bottleneck, we here present a generalized developmental and anatomical ontology to support developmental studies with echinoderms. Among the five echinoderm clades, sea urchins are the most widely used clade, followed by sea stars and then sea cucumbers and ophiuroids, with crinoids the least-studied group. As such, some years ago, an initial Echinoderm Anatomy and Development Ontology (ECAO), covering all developmental stages and some anatomical features of echinoids, from oogenesis to adulthood, was assembled based on knowledge made available by the sea urchin community. The ECAO has been deposited and accepted by the Open Biological and Biomedical Ontology (OBO) Foundry (Smith et al., 2007; Jackson et al., 2021) and can be accessed there (obofoundry.org). In the present study, elaborating on the ECAO, and focusing on the embryogenesis phase (from oogenesis to the opening of the mouth), we have significantly expanded this ontology, refining the developmental stages, and adding new anatomical features. We further analyzed, for all developmental stage and anatomy entity, associated with the embryogenesis phase, their interoperability across five complementary echinoderm species, which together represent the majority of published studies on echinoderm development. These species include four echinoids, three of them (*Lytechinus variegatus*, *Paracentrotus lividus*, and *Strongylocentrotus purpuratus*) belonging to the euechinoid sub-class and one (*Eucidaris tribuloides*) belonging to the cidaroids, which are the sister group to euechinoids. Lastly, we also include one sea star (*Patiria miniata*). By comparing information from these five species, we developed a "pan-echinoderm" developmental and anatomical ontology version of the ECAO subset related to embryogenesis. Taken together, our work provides a comprehensive framework for standardizing, integrating, and curating biological data obtained from studies investigating the embryogenesis of diverse echinoderms.

**Results**

*ECAO ontology design*

The developmental and anatomical ontology ECAO was originally built following the community conventions and best practices recommended by the OBO foundry (Smith et al., 2007). In this data model, each term (or entity), whether it is a developmental stage or an anatomical structure, was automatically assigned a unique IDENTIFIER (ID) (or accession number) in the form of ECAO\_XXXXXXX (Suppl. File 1). Of note, this identifier is uniquely associated with a given term, even if this term is subsequently eliminated or replaced through updates to the ECAO. Therefore, the identifiers do not convey any particular temporal or relationship information and some identifiers, and hence terms, may thus appear as "deprecated" if no longer applicable. Following the OBO foundry recommendations, each term was further provided a generic, or an echinoderm-specific, LABEL (or name), usually in singular but, if mandatory, in plural (Suppl. File 1). This label was chosen, when possible, based on usage of the term in the context of other animal ontologies accessible via the EMBL-EBI Ontology Lookup Service (<https://www.ebi.ac.uk/ols4>), to enable comparisons between echinoderms and other animals as well as to facilitate computer recognition and comprehension by non-specialists. Alternatively, the label was chosen based on the echinoderm literature and corresponding peer-reviewed articles (i.e., CITATIONS) mentioning the related term were provided (Suppl. File 1). Last, if needed, a new, echinoderm-specific label was created. In any event, to further assist users, each developmental stage and anatomical structure was provided with a DEFINITION, describing the main criteria for identifying the related term (Suppl. File 1). Whenever applicable, each ECAO term was also supplemented with SYNONYMS found in the echinoderm literature, and with corresponding UBERON, Cell Ontology (CL), Gene Ontology (GO) or Xenopus Anatomical Ontology (XAO) identifiers (Suppl. File 1), in accordance with the OBO Foundry’s recommended “genus-differentia” format (Smith et al., 2007).

The main additional characteristics of ECAO are its partonomy and developmental grading. The data model used to build ECAO was a directed acyclic graph in which the terms are organized as tree-shaped hierarchies (see example in Fig. 1A, B). Within these hierarchies, general, comprehensive terms are located toward the top of the tree (such as "embryogenesis phase" or "anatomical structure"), while selective terms are positioned toward the bottom (such as "late gastrula stage" or "actin filament") (Fig. 1A, B). In this configuration, ontological relationships (or partonomy) between parent and child terms, whether for developmental stages or anatomical structures, are similarly revealed by either an IS\_A or PART\_OF relationship (Fig. 1C, Suppl. File 1). IS\_A highlights that the child term is a specific type of the parent term. For example, an "archenteron" IS\_A "anatomical structure" (Fig. 1C). Whereas, PART\_OF indicates that the child term is a component of the parent term. In the same example, an "archenteron" is hence PART\_OF "vegetal hemisphere", which is itself PART\_OF "embryo" (Fig. 1C). However, it should be highlighted that a specific entity is often classified by both relationships, IS\_A and PART\_OF, which further provide a hierarchical classification for the IS\_A relationship and a spatial classification for the PART\_OF relationship.

The developmental grading was differently defined for developmental stages and anatomical structures. For developmental stages, the temporal ordering of the stages was exclusively revealed by a PRECEDED\_BY relationship (Suppl. File 1). As an example, the "8-cell stage" is PRECEDED\_BY the "4-cell stage". In contrast, for anatomical structures representation of time was provided by three distinct relationships, which are DEVELOPS\_FROM, STARTS\_AT, and ENDS\_AT (Fig. 1C, Suppl. File 1). Each anatomical structure is thus not only defined in terms of what previous structure it develops from, but also during which developmental stages it exists. Regarding the STARTS\_AT and ENDS\_AT relationships, a structure is pinpointed as STARTS\_AT when it becomes first visible during development and as ENDS\_AT as the last developmental stage at which it is still present. In the example of the term "archenteron" in Figure 1C, an "archenteron" STARTS\_AT the "blastopore formation stage" and ENDS\_AT "late organogenesis stage".

*Extension of ECAO to the embryogenesis of multiple taxa*

In typical indirect-developing echinoderms (the focus of this work), three major phases define the life cycle: the embryogenesis phase, the larval development phase, and the adulthood phase (Fig. 2). The embryogenesis phase is usually considered to be the time period during which the developing organism relies exclusively on maternal sources of energy, i.e., a phase during which there is little or no growth of the organism as a whole. The larval development phase is considered to be characterized by the utilization of external sources of energy, acquired through feeding, to support the growth of tissues and the organism. As such, the transition from the embryogenesis to the larval development phase is marked by the opening of the mouth, the onset of feeding, and the beginning of growth (Gobala Krishnan et al., 2020; Nesbit and Hamdoun, 2020), even though in some indirect-developing echinoderms feeding and growth may be delayed following mouth opening due to a lag in esophageal muscle contractions (e.g. for *P. lividus* - Formery et al., 2022, or *Salmacis sphaeroides* - Rahman et al., 2012). Finally, the adulthood phase is characterized by the fact that the animal is now benthic, lives on the sea floor, and exhibits pentaradial symmetry and an active water vascular system.

ECAO was initially designed to describe all developmental stages and anatomical structures associated with echinoid development from oogenesis to adulthood. It was built essentially from observation and published knowledge from the sea urchin species *P. lividus* and contained about 660 terms. In this study, we have expanded the ECAO to generate a comprehensive dataset aimed at curating in detail the embryogenesis phase of any indirect-developing echinoderm. We have reported on each developmental stage and anatomical structure present during the embryogenesis phase, i.e., from oogenesis to the open mouth stage, in five distinct species, i.e., four echinoids (the euechinoids *P. lividus*, *L. variegatus*, and *S. purpuratus,* and the cidaroid *E. tribuloides*) and one asteroid, *P. miniata*. Our analysis was based on our own observations as well as peer-reviewed literature.

New terms were implemented into ECAO using the same data model that was originally used. For each new term, we thus provided appropriate SYNONYMS, Ontology correspondences, DEFINITION, CITATIONS, partonomy, and developmental grading. Many terms already present in ECAO were also updated in regard to their LABEL, SYNONYMS, Ontology correspondences, DEFINITION, CITATIONS, partonomy, or developmental grading. We further decided to generate a "pan-echinoderm" version of the ECAO subset concerning the embryogenesis phase. For this, we indicated for each new or existing term whether the term is present, absent or not yet reported as present or absent in each of the five echinoderm species we examined or in closely related species (Suppl. File 1\_TaxonDistribution tabs). For developmental stages, we defined the PRECEDED\_BY relationships for each echinoderm species included and for the "pan-echinoderm" staging series (Suppl. File 1\_Dev Period species and Dev\_Pan-Echino tabs). We further provided for each stage a generic definition with taxon-specificities (Suppl. File 2) and a "pan-echinoderm" definition (Suppl. File 1\_Pan-echino tab). For the anatomical structure terms, we provided generic definitions (Suppl. File 1\_Anat. Terms tab) and determined for each species and for the "pan-echinoderm" anatomical series the STARTS\_AT and ENDS\_AT relationships (Suppl. File 1\_Anat. Period species tab). Taken together, the developmental and anatomical ontology related to the ECAO subset dedicated to the embryogenesis phase now comprises a total of 58 developmental stage terms and 622 anatomical terms, each of which is assigned various ontological features and relationships and is present in at least one of the five echinoderm species we examined.

*Developmental staging series*

In this study, we focused on the embryogenesis phase, which has been the subject of most developmental biology studies on echinoderms. Based on direct observations of living embryos (Figs. 3-7, Suppl. Fig. 1), and taking into consideration historical terminology that continues to be widely used by the research community, we defined for each of the five indirectly-developing echinoderm species included in this study an individual embryonic developmental staging series (Fig. 8A, Suppl. File 1)*.* These series were developed with a depth of four nodes (Fig. 8A). The first node encompasses the major life cycle phases: the embryogenesis, larval development, and adulthood phases. The embryogenesis phase was divided into four periods: the cleavage, blastula, gastrula, and organogenesis periods. We further subdivided the gastrula period into two episodes: the mesenchyme blastula and invagination episodes. Finally, each period (or episode) was divided into several embryonic stages, each of which constitutes a terminal node of the hierarchy. For example, this organization led to the assignment of the "8-cell stage" as PART\_OF the "cleavage period", while the "mid-gastrula stage" is PART\_OF the "invagination episode", which is PART\_OF the "gastrulation period" (Fig. 8A).

Within the five developmental staging series established, some stages were species- or taxon-specific. This was the case, for instance, of the "motile blastula stage" specific to *E. tribuloides*, the "very late organogenesis stage" specific to *P. miniata*, and the "early prism stage" specific to *L. variegatus, P. lividus, and S. purpuratus* (Fig. 8A, Suppl. File 1). In addition, because each echinoderm species investigated develops at different temperature and rate, similar stages took place at different times following fertilization. For example, the "8-cell stage" took place at about 2.25 hours post-fertilization (at 22.5°C) for *L. variegatus* but at 4.5 hours post-fertilization (at 15°C) for *P. miniata* (Suppl. Table 1). Moreover, two stages with a similar name could be characterized by different features depending on the echinoderm considered. For instance, the "16-cell stage" was characterized in euechinoids by an embryo composed of three cell tiers exhibiting three distinct cell sizes (Figs. 3-5), while in cidaroids the embryo was composed at this stage of 16 cells of various sizes (Fig. 6), and in asteroids very subtle cell size asymmetry was observed at the 16-cell stage (Fig. 7) (Barone et al, 2022). In summary, the ECAO developmental staging series now includes, in the context of the embryogenesis phase, a total of 39 developmental species-specific stage terms (Suppl. File 1), for each of which a general definition, applicable to all echinoderms, along with taxon-specificities, is provided in Supplemental File 2.

By leveraging the species-specific staging series, we developed a "pan-echinoderm" staging series that integrated the individual stages of all five species into a single series (Fig. 8B,C, Suppl. File 1). This integrated series allows direct, cross-species comparisons and makes possible the digital curation and computational integration of datasets across multiple echinoderm species. A challenge in constructing the "pan-echinoderm" staging series was the variability in the developmental stage at which some developmental events take place across species. These include, for example, the time of hatching, which varies greatly among species (Suppl. File 2). *E. tribuloides* embryos hatch early in development and undergo a prolonged period as a swimming blastula before the onset of gastrulation (Fig. 6). By contrast, most euechinoid embryos, including *L. variegatus* and *P. lividus*, hatch just before the ingression of skeletogenic primary mesenchyme cells (PMCs), an event that marks the onset of gastrulation (Figs. 3,4). Yet, in the euechinoid *S. purpuratus*, hatching is usually delayed until after PMC ingression is underway (Fig. 5). There is also significant interspecies variability in the timing of appearance of mesenchyme cells, which occurs prior to the invagination of the vegetal plate in euechinoid sea urchins but much later in gastrulation in cidaroids and asteroids (Figs. 3-7, Suppl. Files 1 and 2). Likewise, the formation of the hydropore canal rudiment occurs concomitantly with the opening of the mouth in *S. purpuratus, E. tribuloides*,and *P. miniata* (Figs. 5-7, Suppl. Files 1 and 2)*,* but after the onset of feeding in *L. variegatus* and *P. lividus* (Figs. 3,4, Suppl. Fig. 1) (Formery et al., 2022). Interspecies variability in the timing of these developmental events precluded thus their use as reference points for the "pan-echinoderm" staging series.

Nonetheless, many other major features of embryonic development were shared by all the indirectly developing echinoderms we studied and could be used to ground the "pan-echinoderm" staging series. For example, in all echinoderms that have been examined, the zygote undergoes initially two holoblastic cleavages and gives rise eventually to a spherical, ciliated blastula with a central blastocoel (Figs. 3-7). Gastrulation is preceded by the formation of a thickened vegetal plate which then invaginates, and mesenchymal cells are released from the tip of the archenteron as it elongates (Figs. 3-7). The primitive gut undergoes an anterior-to-posterior wave of morphogenesis that begins with an expansion of the anterior tip followed by the outpocketing of paired (left and right) coelomic pouches (Figs. 3-7). Gut morphogenesis continues with the formation of two constrictions that give rise in the larva to two sphincters: first the cardiac constriction at the foregut-midgut boundary and then the pyloric constriction at the midgut-hindgut boundary (Figs. 3-7). Concomitant with later events of gut morphogenesis, an invagination of the oral ectoderm also commonly produces the stomodeum, and morphogenetic interactions between the foregut and the stomodeal invagination bring these two tissues together, leading to the opening of the mouth and the onset of feeding (Figs. 3-7). We thus used all these universal features of echinoderm indirect development to anchor the "pan-echinoderm" staging series, which includes 19 stages and is depicted in Figure 8 and Supplemental Table 1 and File 1.

*Anatomical series*

Anatomical ontologies consist of a computable representation of the parts, structures, and elements of a developing organism. Based on direct observations of living embryos (Figs. 3-7, Suppl. Fig. 1) and taking into consideration available information from published literature, we next assembled a comprehensive list of anatomical entities present during the embryogenesis phase of the five indirectly-developing echinoderm species we examined (Suppl. File 1)*.* This list includes gamete-related entities, embryo-specific entities, and embryonic entities that continue to be present during the larval development and adulthood phases of the life cycle. The anatomical series was developed with a depth of several nodes, with the anatomical structure, system, region, space, and element representing the highest-level of the nodes and providing the starting points for a structural classification scheme (Fig. 1B). Obviously, not all anatomical entities were subject to the same node depth. For example, a general entity, such as "archenteron", had a lesser node depth then a more specific entity, such as the "right coelomic pouch" or "actin filament" (Fig. 1B, Suppl. File 1). In these examples, "archenteron" IS\_A "anatomical structure" (Fig. 1C), while the "right coelomic pouch" IS\_A "coelomic pouch", which itself IS\_A "coelom" that IS\_A "anatomical structure" (Suppl. File 1).

Similar to the developmental staging series, the taxonomic distribution of anatomical entities, across the five species included in this study, also revealed some species- or taxon-specificity. For example, the "equatorial pigment band" has only been reported to date in the euechinoid sea urchin *P. lividus*, and the "preoral ciliary band" and "postoral ciliary band", which only applies to bilaterian larvae with two separate ciliary bands, are only present in the sea star *P. miniata* and other sea stars (Suppl. File 1). The term "micromere" designates a cell type found only in all euechinoids, and not in cidaroids or asteroids (Suppl. File 1). Similarly, an elaborate, calcified "endoskeleton" is present in echinoids (both euechinoids and cidaroids), but is entirely absent from asteroids, while a "posterior enterocoel" forms in *P. miniata* and other asteroids but is absent from euechinoids and cidaroids (Suppl. File 1). Several other structures have also been described in euechinoids, such as "bottle cell", "endoderm-associated neuron", and sub-classes of blastocoelar cells, but these have not yet been reported in other, less well-studied, echinoderm taxa, precluding any conclusions about their presence or absence in these taxa. As a consequence, the anatomical series we developed as part of the ECAO subset related to the embryogenesis phase encompasses to date a total of 622 entities, for each of which partonomy, developmental, and species attributes are provided in Supplemental File 1.

Using the species-specific developmental staging series we constructed, we also defined, in each of the five species included in this study, the developmental window during which each entity is present. While doing so, we noted some variability in the timing of appearance and/or disappearance of some anatomical entities, as reflected in Supplemental File 1. For example, the "presumptive ciliary band", a region of the embryo marked by the onset of *onecut* expression, is first apparent at the mesenchyme blastula stage in *S. purpuratus* (Poustka et al, 2004; Barsi and Davidson, 2016), but does not arise until well after the onset of gastrulation in *P. miniata* (Yankura et al., 2013) and *E. tribuloides* (Bishop et al., 2013). Likewise, "germ cell" emerges at the 32-cell stage in euechinoids such as *S. purpuratus,* while this cell type becomes detectable at a molecular level only at the late organogenesis stage in *P. miniata* (Fresques et al., 2014). While some of this interspecies variability is likely to be genuine, some may be attributable to variations in the experimental methods used in published studies and/or differences in the granularity of the analyses, thereby reinforcing the need to establish echinoderm anatomical ontologies.

Despite the variability observed across species, the taxonomic distribution of the anatomical entities revealed that the great majority of the reported entities were found throughout the three echinoderm clades included in this study. More specifically, of all entities encompassed in the embryogenesis phase subset of the ECAO anatomical series, more than 97% were present in all three euechinoid species we examined, 93% were present in *E. tribuloides*, and 49% were present in *P. miniata* (Suppl. File 1). In the latter case, the differences were mostly due to the lack of a skeleton in asteroid embryos. Moreover, most of the common entities have also been reported in holothuroids and ophiuroids, the two other echinoderm classes that exhibit indirect development. This is the case, for example, with regard to entities such as "apical ganglion", "archenteron", "cilium", and "stomodeum". In addition, in most cases, the time period during which each of these shared entities was present was similar across species (Suppl. File 1). These features thus reflected the evolutionary conservation of developmental processes across the phylum and enabled the construction of a "pan-echinoderm" anatomical series (Suppl. File 1\_Anat. Period species tab). Concerning both the set of anatomical entities and the time frame during which they are present in each echinoderm species we examined, we thus developed a "pan-echinoderm" anatomical series, for which we employed a 'union' model. This means that any anatomical entity present in at least one of the five species was included in the "pan-echinoderm" set of entities. Similarly, the time frame during which an entity was present in the "pan-echinoderm" anatomical series utilized the earliest STARTS\_AT stage for that entity in any of the five species and the latest ENDS\_AT stage for that entity in any of the five species. As such, the "pan-echinoderm" anatomical series developed here (Suppl. File 1) is providing for the first time a unique tool that will be key to curate, annotate, and compare gene expression patterns and phenotypes across echinoderms.

**Methods**

*ECAO ontology*

ECAO was originally built in the open source graphical ontology editor OBO-Edit (Day-Richter et al., 2007) and is continuously curated and updated. Requests for modifications may be submitted to the corresponding authors or through Echinobase. ECAO is available as OBO and Web Ontology Language (OWL) format files through GitHub (https://github.com/echinoderm-ontology/ecao\_ontology). It is also available in the same formats through the OBO foundry website (https://obofoundry.org/ontology/ecao.html) (Jackson et al., 2021) and for user searches through the EMBL-EBI Ontology Lookup Service (https://www.ebi.ac.uk/ols4/ontologies/ecao). Soon, it will also become available for downloads and user searches at Echinobase (https://www.echinobase.org/echinobase/) (Arshinoff et al., 2022; Telmer et al, 2024) and Marimba (http://marimba.obs-vlfr.fr/).

All ECAO terms that were generated as part of this study, along with related synonyms, partonomy, developmental grading, definitions, time frames, and presence or absence in each of the five echinoderm species examined, were determined from direct observations made during this study or from published peer-reviewed articles. Whenever possible, developmental stage and anatomical term labels and definitions were adopted from those found in UBERON (Mungall et al., 2012; Haendel et al., 2014). The information was compiled and formatted into a single Excel file containing separate tabs for the developmental staging series and the anatomical entities (Suppl. File 1). The "pan-echinoderm" developmental staging and anatomical ontology was built from the data collected from the five echinoderm species investigated, using a 'union' model; the "pan-echinoderm" stage range for each anatomical entity uses the earliest STARTS\_AT stage for that entity in any of the five species and the latest ENDS\_AT stage in any of the five species.

*Animals*

Five representative, indirect-developing echinoderms were used: three euechinoids (*Lytechinus variegatus*, *Paracentrotus lividus*, *Strongylocentrotus purpuratus*), one cidaroid (*Eucidaris tribuloides*) and one asteroid (*Patiria miniata*). Gravid adult sea urchins and sea stars were obtained from Patrick Leahy (California Institute of Technology, Pasadena, CA, USA) (*S. purpuratus* and *P. miniata*), Pelagic Corp. (Sugarloaf Key, FL, USA) (*E. tribuloides* and *L. variegatus*), and the IMEV Service Aquariologie (Institut de la Mer de Villefranche, Villefranche-sur-Mer, France) (*P. lividus*). For each species, light microscopic observations were carried out on at least four batches of embryos derived from separate mating pairs. Embryos were cultured in glass bowls or 6 to 10 cm plastic cell culture dishes, without stirring or feeding, at 15°C (*P. miniata* and *S. purpuratus*), 18°C (*P. lividus*), or 22.5° C (*E. tribuloides* and *L. variegatus*) using temperature-controlled incubators.

*Imaging*

For light microscopic observations, at the appropriate developmental time following fertilization, embryos were concentrated by low-speed centrifugation (*E. tribuloides*, *L. variegatus, P. miniata,* and *S. purpuratus*)or collected by micropipetting (*P. lividus*) and mounted on glass slides. To prevent compression of the embryos, coverslips were supported by spacers that consisted of one or more layers of double-sided tape (Scotch #665, 3M Corp., St. Paul, MN, USA) or clay. For examination of post-hatching (swimming) stages, embryos that had been collected by centrifugation were re-suspended in 0.01% protamine sulfate (Cat. P4020, Sigma-Aldrich, Burlington, MA, USA) diluted in sea water to slow ciliary beating and then mounted on coverslips that had been coated with poly-L-lysine hydrobromide (Ettensohn, 1985) (*E. tribuloides*, *L. variegatus, P. miniata,* and *S. purpuratus*). For embryos that were collected by micropipetting, they were immobilized by adding to the cell culture dish a drop of 8% paraformaldehyde (Cat. P6148, Sigma-Aldrich, Saint-Quentin-Fallavier, France) prepared in sea water (*P. lividus*). Mounted specimens were examined with a 20x dry or 40x oil immersion objective using differential interference contrast (DIC) optics.

For counts of cell numbers at late cleavage stages, embryos were fixed in 100% methanol overnight at -20°C, rinsed five times in PBS 1x, and stained for 10 minutes at room temperature with 10 g/ml Hoechst 33342 (Cat. H3570, Invitrogen, Carlsbad, CA, USA) diluted in PBS 1x. Squash preparations were imaged using epifluorescence optics and nuclei were counted.

All images were collected with either a LC30 CMOS color camera (Olympus Corp. Tokyo, Japan) and a Xyla 4.2 sCMOS camera (Oxford Instruments, Abingdon, UK) (*E. tribuloides*, *L. variegatus, P. miniata,* and *S. purpuratus*) or an Axiocam 506 Color camera (Zeiss, Jena, Germany) (*P. lividus*). All acquired images were processed with cellSens imaging software (Olympus Corp. Tokyo, Japan) and Adobe Photoshop (Adobe, Inc., San Jose, CA, USA) (*E. tribuloides*, *L. variegatus, P. miniata,* and *S. purpuratus*), or with ImageJ version 1.44o (Schneider et al., 2012) and Affinity Photo (Serif, Nottingham, United Kingdom) (*P. lividus*).

**Discussion**

Echinoderms, and in particular sea urchins and sea stars, have been prominent model organisms for developmental biology studies for more than a century. Despite this rich history, no ontology of embryogenesis has been produced for any echinoderm, although atlases of development have recently been published for three euechinoids, *Lytechinus variegatus* (Morrill and Markus, 2005), *Paracentrotus lividus* (Formery et al., 2022), and *Lytechinus pictus* (Nesbit and Hamdoun, 2020). In the absence of a formal ontology, the interpretation of widely used terms such as “early gastrula" and “prism" varies greatly, even among experts. As a consequence, it is very difficult to compare experimental data across studies, even within a single species. The rapid accumulation of biological data of many types, including mRNA expression patterns, protein localization data, and embryonic phenotypes resulting from molecular, pharmacological, and genetic manipulations has also created a need for tools to support the curation and integration of this information. The ontology we present here will provide a framework for the digital curation of these important types of experimental data by knowledgebases such as Echinobase, the knowledgebase of echinoderm gene-related information (Arshinoff et al., 2022: Telmer et al., 2024) and Marimba, the knowledgebase of invertebrate marine models (http://marimba.obs-vlfr.fr/). More generally, the ECAO developmental and anatomical ontology will significantly enhance the utility of echinoderms for biological research, by standardizing experimental data, increasing reproducibility, and facilitating comparisons and integration of information across laboratories, experiments, and species.

One important application of developmental staging series and anatomical series is to describe gene expression patterns and phenotypes with a controlled, fixed vocabulary, facilitating comparisons between normal and experimentally perturbed embryos as well as between species (Bradford et al., 2023; Fisher et al., 2023; Öztürk-Çolak et al., 2024). In some model systems, anatomical series have been used to characterize gene expression patterns in wild-type embryos, genetic mutants and embryos that have been treated with pharmacological inhibitors or following targeted gene knockdowns (Finger et al., 2011; Knowlton et al., 2008). Likewise, in those model systems, anatomical series have been employed to describe phenotypes of wild-type embryos, genetic mutants, and embryos following gene function perturbations (Beck et al., 2009; Osumi-Sutherland et al., 2013). These series and their use are therefore providing a way to annotate gene expression patterns and phenotypes in a form that is computationally accessible and query-able, and that can be used to group annotations in biologically meaningful ways. Based on these considerations, we anticipate that a major application of the ontology we have produced will be the curation on Echinobase and Marimba of gene expression patterns, both during normal development and after experimental perturbations, as well as the curation of presence and absence or alteration of anatomical structures in respectively control and experimental perturbation conditions.

Another valuable application of the developmental stage and anatomical series we produced will be the annotation of the expression patterns controlled by transcriptional drivers. Cis-regulatory elements (CREs) that drive transgene expression in specific spatiotemporal patterns are used by the echinoderm research community to control the expression of diverse molecules, including transcription factors, Cas9, inducible transcriptional activators (e.g., tTA), and fluorescent cell lineage markers (Damle and Davidson, 2012; Barsi et al., 2014; Cheatle-Jarvela et al., 2016; Khor and Ettensohn, 2023). By using the ontology developed here to annotate CRE expression patterns, the research community will have access to a comprehensive, searchable palette of transcriptional drivers for directing transgene expression to specific cell types or tissues.

We deliberately based our developmental staging scheme here on morphological landmarks that can easily be observed in living embryos using only a light microscope. Conventional bright-field imaging is sufficient in this regard, although differential contrast (DIC) imaging is useful. The only developmental stages that are difficult to identify in this manner are the early blastula to late blastula stages, when there are no anatomical changes in the spherical embryo other than an increase in cell number and a concomitant decrease in cell size, and when there are too many cells to count easily. To accurately stage such embryos, the most reliable approach is to label embryos with nuclear stains such as DAPI or Hoechst dye and count cell numbers in squash preparations. Other researchers have opted, for *P. lividus* and *L. variegatus* in particular, to sequentially name these stages one after the other with a time interval in between each of them of one hour of development (Croce and McClay, 2010, Formery et al., 2022). However, this approach is restricted to a standard temperature and is difficult to extend across species.

In this work, we have necessarily limited the number of echinoderm species we examined. The five indirectly developing species that we incorporated into the current ontology were chosen because they account for most published studies on echinoderm development and represent diverse developmental patterns and deeply divergent clades within the phylum. Among euechinoids, a recent (February, 2025) search of PubMed entries with the terms “<species name>" and "embryo” indicates that the three most widely used species are *S. purpuratus* (837 citations), *P. lividus* (586 citations), and *L. variegatus* (384 citations). The next-most widely cited species, the sea urchins *H. pulcherrimus* (220 citations) and *L. pictus* (186 citations), are very closely related to *S. purpuratus* and *L. variegatus*, respectively. It should thus be straightforward to apply our ontology to these species with few modifications. In support of this view, a developmental atlas for *L. pictus* has been published (Nesbit and Hamdoun, 2020), and the embryonic stages of this species map in a very straightforward way to the stages we describe for *L. variegatus*. Aside from euechinoids, our study did not include ophiuroids (brittle stars) or holothuroids (sea cucumbers) because their embryos are not presently widely used for developmental biology studies, although these taxa are extremely valuable from a comparative perspective. Similarly, our focus on species that undergo maximally indirect development inevitably excluded consideration of crinoids, as all species of crinoids that have been described to date exhibit direct development. However, our study included a cidaroid (*E. tribuloides*) and an asteroid (*P. miniata*), along with the more commonly used euechinoids, which enabled us to show that a single ontology can be applied to deeply divergent echinoderms and to establish a foundation for extending the ontology to additional species in the future.

The growing interest in comparative studies using echinoderms has indeed created a need for an ontology that can integrate biological information across the phylum. This is why we have made an effort to produce a "pan-echinoderm" ontology. At present, our "pan-echinoderm" developmental stage and anatomical series are based on data from five distinct echinoderm species that exhibit maximally indirect development and, further, to the embryonic phase of indirect development, which culminates in the opening of the mouth and the beginning of feeding and growing. These limitations seem justified, as most modern echinoderms exhibit indirect development and the vast majority of published studies have examined the embryonic phase of indirect development. Nonetheless, comparisons with species that undergo direct development, an evolutionary derived mode that has arisen independently multiple times (Wray, 2022), would be highly informative, even though the embryogenesis of such species is often so radically different from that of indirect developers that the creation of a developmental and anatomical ontology encompassing both direct and indirect developing species will certainly present a significant challenge for the future.

The diversity of developmental patterns among echinoderms presented already a challenge to the creation of a single, unified ontology. Even when comparing different species of euechinoids (*L. variegatus*, *P. lividus* and *S. purpuratus*) we identified several differences, and these were even more pronounced when comparing a euechinoid to a cidaroid or an asteroid species. Some of these apparent interspecies differences may be attributable to variability in the experimental methods that have been used and/or to differences in the granularity of the analyses. Nevertheless, our evaluation of the embryogenesis of the five species we included in this study enabled us to define a "pan-echinoderm" developmental staging series and many "pan-echinoderm" anatomical structures. In fact, our work appears to be among the first attempts to integrate both the developmental and anatomical ontologies of such diverse and deeply divergent species, even though multi-species developmental ontologies have started to be produced (Carvalho et al., 2021; Mungall et al., 2022). As such, this work will certainly prove to be the stepstone of more integrative animal ontologies in the future and will already enable precise curation, annotation, and comparisons across studies carried out on echinoderms.

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**Figure Captions**

**Figure 1. Schematic representations of ontological relationships in ECAO.** (A) Example of developmental staging relationships. Lines between the different depth nodes correspond to PART\_OF relationships. (B) Example of anatomical entity relationships. Dotted lines between the different terms represent IS\_A relationships. (C) Example of another ontological representation for the anatomical entity "archenteron". This representation includes the partonomy relationships of the entity (IS\_A, PART\_OF, DEVELOPS\_FROM) and that of some of its related terms. It also includes the developmental stages during which the entity exists (STARTS\_AT and ENDS\_AT).

**Figure 2. Major stages in the life cycle of a representative, indirect-developing echinoderm.** Indirect development is the most common and ancestral life history strategy within the echinoderm phylum. Diagrams show euechinoid larvae and adults for illustrative purposes and are not drawn to scale. Fertilization occurs externally and is followed by a short embryogenesis phase (1-4 days) during which the zygote develops rapidly into a swimming, feeding larva. The opening of the mouth, onset of feeding, and beginning of growth mark the starting point of a longer period of larval development (typically several weeks in length), during which the larva grows in size and develops new anatomical structures. Of these, a key event during this phase of the life cycle is the development of the echinus adult rudiment, the progenitor of the juvenile, which forms on the left side of the larval stomach from the left coelom and an invagination of the ectoderm (the vestibule). At metamorphosis, the larva settles on a substrate and a benthic juvenile emerges from the degenerating larval body. This sets forth the beginning of the adulthood phase (typically several years in length) during which the juvenile grows (usually over several months after metamorphosis) before it becomes sexually mature. The life cycle is then repeated.

**Figure 3. Embryonic stages of the euechinoid *Lytechinus variegatus*.** Stages correspond to those indicated in Figure 8 and Supplemental Files 1 and 2. Images of early embryonic stages (from 1-cell to LG) include only lateral views, with the animal pole up. Images of late embryonic stages (from EPr to OM) include anterior (a), lateral (l), posterior (p) and ventral (v) views. Images labeled (a1) and (a2) show the same embryo viewed in two different focal planes in anterior view. Stage abbreviations (in time order): 1-cell, 1-cell stage; 2-cell, 2-cell stage; 4-cell, 4-cell stage; 8-cell, 8-cell stage; 16-cell, 16-cell stage; 28-cell, 28-cell stage; 32-cell; 32-cell stage; 56-cell, 56-cell stage; EB, early blastula stage; MB, mid-blastula stage; LB, late blastula stage; VP, vegetal plate stage; EMesB, early mesenchyme blastula stage; MMesB, mid-mesenchyme blastula stage; LMesB, late mesenchyme blastula stage; BF, blastopore formation stage; EG, early gastrula stage; MG, mid-gastrula stage; LG, late gastrula stage; EPr, early prism stage; LPr, late prism stage; LO, late organogenesis stage; OM, open mouth stage. Other abbreviations: AN, anus; AP, animal pole domain; AR, archenteron; BC, blastocoel; BP, blastopore; CB, ciliary band; CC, cardiac constriction; CEs, coelomic evaginations; CPs, coelomic pouches; E, esophagus; FE, fertilization envelope; M, mouth; Mac, macromere; Mes, mesomere; Mic, micromere; OH, oral hood; P, pigment cell; PMCs, primary mesenchyme cells; POAs, postoral arms; S, stomach; SK, skeletal rod; SMCs, secondary mesenchyme cells; sMic, small micromere; ST, stomodeal invagination; VgP, vegetal plate. Scale bar = 100 m.

**Figure 4. Embryonic stages of the euechinoid *Paracentrotus lividus.*** Stages correspond to those reported in Figure 8 and Supplemental Files 1 and 2. Images of early embryonic stages (from 1-cell to LG) include only lateral views, with the animal pole up. Images of late embryonic stages (from EPr to OM) include anterior (a), lateral (l), posterior (p) and ventral (v) views. Images labeled (l1) and (l2) show the same embryo viewed in two different focal planes in lateral view. Stage abbreviations (in time order): 1-cell, 1-cell stage; 2-cell, 2-cell stage; 4-cell, 4-cell stage; 8-cell, 8-cell stage; 16-cell, 16-cell stage; 32-cell; 32-cell stage; 60-cell, 60-cell stage; EB, early blastula stage; MB, mid-blastula stage; LB, late blastula stage; VP, vegetal plate stage; EMesB, early mesenchyme blastula stage; MMesB, mid-mesenchyme blastula stage; LMesB, late mesenchyme blastula stage; BF, blastopore formation stage; EG, early gastrula stage; MG, mid-gastrula stage; LG, late gastrula stage; EPr, early prism stage; LPr, late prism stage; LO, late organogenesis stage; OM, open mouth stage. Other abbreviations: AN, anus; AP, animal pole domain; AR, archenteron; BC, blastocoel; BP, blastopore; CB, ciliary band; CC, cardiac constriction; CEs, coelomic evaginations; CPs, coelomic pouches; E, esophagus; FE, fertilization envelope; M, mouth; Mac, macromere; Mes, mesomere; Mic, micromere; OH, oral hood; P, pigment cell; PC, pyloric constriction; PMCs, primary mesenchyme cells; POAs, postoral arms; S, stomach; SK, skeletal rod; SMCs, secondary mesenchyme cells; sMic, small micromere; ST, stomodeal invagination; VgP, vegetal plate. Scale bar = 100 m.

**Figure 5. Embryonic stages of the euechinoid *Strongylocentrotus purpuratus*.** Stages indicated correspond to those in Figure 8 and Supplemental Files 1 and 2. Images of early embryonic stages (from 1-cell to LG) include only lateral views, with the animal pole up. Images of late embryonic stages (from EPr to OM) include anterior (a), dorsal (d), lateral (l), and ventral (v) views. Images labeled (l1) and (l2) show the same embryo viewed in two different focal planes in lateral view. Stage abbreviations (in time order): 1-cell, 1-cell stage; 2-cell, 2-cell stage; 4-cell, 4-cell stage; 8-cell, 8-cell stage; 16-cell, 16-cell stage; 28-cell; 28-cell stage; 56-cell, 56-cell stage; EB, early blastula stage; MB, mid-blastula stage; LB, late blastula stage; VP, vegetal plate stage; EMesB, early mesenchyme blastula stage; MMesB, mid-mesenchyme blastula stage; LMesB, late mesenchyme blastula stage; BF, blastopore formation stage; EG, early gastrula stage; MG, mid-gastrula stage; LG, late gastrula stage; EPr, early prism stage; LPr, late prism stage; LO, late organogenesis stage; OM, open mouth stage. Other abbreviations: AN, anus; AP, animal pole domain; AR, archenteron; BC, blastocoel; BP, blastopore; CC, cardiac constriction; CEs, coelomic evaginations; CPs, coelomic pouches; E, esophagus; FE, fertilization envelope; HC, hydropore canal rudiment; HL, hyaline layer; M, mouth; Mac, macromere; Mes, mesomere; Mic, micromere; OH, oral hood; PC, pyloric constriction; PMCs, primary mesenchyme cells; POA, postoral arm; S, stomach; SK, skeletal rod; SMCs, secondary mesenchyme cells; sMic, small micromere; VgP, vegetal plate. Scale bar = 50 m.

**Figure 6. Embryonic stages of the cidaroid *Eucidaris tribuloides*.** Stages correspond to those reported in Figure 8 and Supplemental Files 1 and 2. Images of early embryonic stages (from 1-cell to EO) include only lateral views, with the animal pole up. Images of late embryonic stages (from MO to OM) include lateral (l) and ventral (v) views. Stage abbreviations (in time order): 1-cell, 1-cell stage; 2-cell, 2-cell stage; 4-cell, 4-cell stage; 8-cell, 8-cell stage; 16-cell, 16-cell stage; 32-cell; 32-cell stage; 64-cell, 64-cell stage; EB, early blastula stage; MotB, motile blastula stage; LB, late blastula stage; VP, vegetal plate stage; BF, blastopore formation stage; EG, early gastrula stage; MG, mid-gastrula stage; LG, late gastrula stage; EO, early organogenesis stage; MO, mid-organogenesis stage; LO, late organogenesis stage; OM, open mouth stage. Other abbreviations: AR, archenteron; BC, blastocoel; BP, blastopore; CB, ciliary band; CC, cardiac constriction; CEs, coelomic evaginations; CPs, coelomic pouches; E, esophagus; FE, fertilization envelope; HC, hydropore canal rudiment; M, mouth; Mac, macromere; MC, mesenchyme cell; Mes, mesomere; Mic, micromere; OH, oral hood; P, pigment cell; PC, pyloric constriction; S, stomach; SK, skeletal rod; SM, skeletogenic mesenchyme; ST, stomodeal invagination; VgP, vegetal plate. Scale bar = 100 m.

**Figure 7. Embryonic stages of the asteroid (sea star) *Patiria miniata.***Stages correspond to those in Figure 8 and Supplemental Files 1 and 2. Images of early embryonic stages (from 1-cell to EO) include only lateral views, with the animal pole up. Images of late embryonic stages (from MO to OM) include dorsal (d), lateral (l) and ventral (v) views. Stage abbreviations (in time order): 1-cell, 1-cell stage; 2-cell, 2-cell stage; 4-cell, 4-cell stage; 8-cell, 8-cell stage; 16-cell, 16-cell stage; 32-cell; 32-cell stage; 64-cell, 64-cell stage; EB, early blastula stage; MB, mid-blastula stage; LB, late blastula stage; VP, vegetal plate stage; BF, blastopore formation stage; EG, early gastrula stage; MG, mid-gastrula stage; LG, late gastrula stage; EO, early organogenesis stage; MO, mid-organogenesis stage; LO, late organogenesis stage; VLO, very late organogenesis; OM, open mouth stage. Other abbreviations: AR, archenteron; BC, blastocoel; BP, blastopore; CBs, ciliary bands; CC, cardiac constriction; CEs, coelomic evaginations; CPs, coelomic pouches; E, esophagus; FE, fertilization envelope; HC, hydropore canal rudiment; M, mouth; MC, mesenchyme cell; OH, oral hood; PE, posterior enterocoel; PC, pyloric constriction; S, stomach; ST, stomodeal invagination; VgP, vegetal plate. Scale bar = 100 m.

**Figure 8. Embryonic staging series for five representative, indirect-developing echinoderm species and correspondence with the "pan-echinoderm" staging series.** (A) The species-specific embryonic staging series are depicted following the four developmental ontology depth nodes (phase, period, episode, stage). The five representative, indirect-developing echinoderm species include three euechinoid sea urchins (*Lytechinus variegatus*, *Paracentrotus lividus*, and *Strongylocentrotus purpuratus*), one cidaroid sea urchin (*Eucidaris tribuloides*), and one sea star (*Patiria miniata*). (B, C) The "pan-echinoderm" staging series is shown in the form of a table (B) and a graph (C). It integrates the five individual schemes into a single series applicable to all echinoderms. Detailed definitions of all developmental phases, periods, episodes, and stages are provided in Supplemental Files 1 and 2.

**Supplemental File 1. Table with all developmental stages and anatomical terms related to the embryogenesis phase of euechinoid, cidaroid, asteroid, and "pan-echinoderm" indirect developers.** The table encompasses a total of eight tabs. Tab 1 includes the 39 developmental stages related to *Lytechinus variegatus*, *Paracentrotus lividus*, *Strongylocentrotus purpuratus*, *Eucidaris tribuloides* and *Patiria miniata* embryogenesis phase and reported in Figure 8. It displays the partonomy relationships between the stages (IS\_A, PART\_OF, PRECEDED\_BY) as well as synonyms, Uberon correspondences, and PubMed references citing the associated developmental stage in a non-exhaustive way. Tab 2 reports on the distribution of the 39 developmental stages in each of the five echinoderm species used in this study and their associated taxon. Tab 3 highlights the temporal ordering of the stages in each of the five echinoderm species investigated. Grey cells indicate absence of the corresponding stage in the related animal. Tab 4 exposes the "pan-echinoderm" staging series including the partonomy relationships between the stages (IS\_A, PART\_OF, PRECEDED\_BY) along with a proper definition for each stage. Tab 5 outlines the PART\_OF relationship between each species-specific developmental stage and the "pan-echinoderm" stages. Tab 6 encloses the 622 anatomical entities related to the embryogenesis phase of *L. variegatus*, *P. lividus*, *S. purpuratus*, *E. tribuloides* and *P. miniata*. It further includes the partonomy relationships between the entities (IS\_A, PART\_OF, DEVELOPS\_FROM), along with synonyms, Uberon correspondences, PubMed references citing the associated entity in a non-exhaustive way, and a definition for each entity. Tab 7 reports on the distribution of each of the 622 anatomical entities in each of the five echinoderm species included in the study and/or their related taxon. Text in black indicates reported presence of the corresponding entity in the related animal, text in blue reported absence of the corresponding entity in the related animal, and text in brown reported presence of the corresponding entity not in the studied species but in one species of the same taxon. "-" indicates no reports yet about the presence or absence of the corresponding entity in any species of the related taxon. Tab 8 indicates for each anatomical entity the developmental stages during which the entity is present (STARTS\_AT and ENDS\_AT) in each of the five echinoderm species looked at and/or their related taxon as well as how this translates in the context of the "pan-echinoderm" developmental staging series. Grey cells highlight reported absence of the corresponding entity in the related animal. Text in brown indicates that information arises from another species than that studied, but from the same taxon. "-" marks entities for which the absence or presence in the related animal has not yet been reported. "?" outlines that the entity exists in the related animal, but the developmental stages during which it is present remain unknown. For the "pan-echinoderm" entries, we followed a 'union' model, meaning that the "pan-echinoderm" stages span the widest range of the various individual species stages (from the earliest START\_AT to the latest ENDS\_AT).

**Supplemental File 2. Definitions of developmental phases, periods, episodes, and stages that apply to euechinoid, cidaroid, and asteroid indirect developers.** The definitions are provided for all terms included in Figure 8 and Supplemental File 1. For each phase, period, episode, and stage, a general definition is given that is universally applicable to euechinoid, cidaroid, and asteroid indirect developers. However, when needed, taxon-specificities are further provided.

**Supplemental Figure 1. Late embryonic stages of the euechinoid *Lytechinus variegatus*.** High magnification views illustrating (A) archenteron morphogenesis, (B) skeletogenesis, and (C) other features of late embryogenesis. Stages correspond to those indicated in Figure 8 and described in Supplemental Files 1 and 2. Images include anterior (a), lateral (l), posterior (p), and ventral (v) views. Images labeled (v1) and (v2) or (a1) and (a2) show the same embryo viewed in two different focal planes, respectively in ventral and anterior views. In (A), arrowhead indicates the smooth anterior tip of the archenteron and asterisks mark the expanded tip of the archenteron. In (B), arrowhead points to a tri-radiate spicule rudiment and asterisk indicates the position at the ventral midline where the tips of two ventrolateral transverse rods meet. Stage abbreviations (in time order): MG, mid-gastrula stage; LG, late gastrula stage; EPr, early prism stage; LPr, late prism stage; LO, late organogenesis stage; OM: open mouth stage. Other abbreviations: A, anonymous rod; AL, anterolateral rod; AN, anus; AR, archenteron; B, body rod; CB, ciliary band; CC, cardiac constriction; CP, coelomic pouch; DV, dorsoventral connecting rod; E, esophagus; FG, foregut; HG, hindgut; M, mouth; MIG, midgut; P, pigment cell; PC, pyloric constriction; PO, postoral rod; R, recurrent rod; S, stomach; SC, scheitel; SMCs, secondary mesenchyme cells; ST, stomodeal invagination; VT, ventral transverse rod. Scale bars = 50 m.

**Supplemental Table 1. Timescale comparison of developmental timing for each of the five species included in the study and correspondence with the "pan-echinoderm" stages.** Each species develops at a typical culture temperature. Embryos were thus cultured, without stirring and feeding, at 15°C for *P. miniata* and *S. purpuratus*, 18°C for *P. lividus*, and 22.5° C for *E. tribuloides* and *L. variegatus*, using temperature-controlled incubators. Entries indicate the time (in hours) after fertilization at which embryos reached the indicated stages. Times should however be considered approximate as there is some variability in developmental rate both within and between cultures. Text in black highlights stages common to all species, while text in grey indicates species- or taxon-specific stages. Data are based on observations of at least four cultures of each species, each derived from a separate mating pair. Additional information concerning the timing of *S. purpuratus*, *P. lividus*, *E. tribuloides*, and *L. variegatus* development can respectively be found in Harrison and Wilt (1982), Formery et al. (2022), Schroeder (1981), and Morrill and Marcus (2005). The last column on the right highlights the correspondence with the "pan-echinoderm" stages. Definitions of each species and "pan-echinoderm" developmental stage are provided, respectively, in Supplemental Files 2 and 1. Abbreviations: 28-cell, 28-cell stage; 32-cell, 32-cell stage; 56-cell, 56-cell stage; 60-cell, 60-cell stage; 64-cell, 64-cell stage; e.mes., early mesenchyme blastula stage; m.mes., mid-mesenchyme blastula stage; l.mes., late mesenchyme blastula stage; blasto., blastopore formation stage; l.orga., late organogenesis stage; v.l.orga., very late organogenesis stage.