BOLD AWARD

BENDING STRATEGIES OF CONVERGENTLY EVOLVED, ARTICULATED CORALLINE ALGAE[1]

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The evolution of uncalcified genicula in upright calcified corallines has occurred at least three times independently, resulting in articulated corallines within Corallinoideae, Lithophylloideae, and Metagoniolithoideae. Genicula confer flexibility to otherwise rigid thalli, and the localization of bending at discrete intervals amplifies bending stress in genicular tissue. Genicular morphology must, therefore, be balanced between maintaining flexibility while mitigating or resisting stress. Genicula in the three articulated lineages differ in both cellular construction and development, which may result in different constraints on morphology. By studying the interaction between flexibility and morphological variation in multiple species, we investigate whether representatives of convergently evolving clades follow similar strategies to generate mechanically successful articulated fronds. By using computational models to explore different bending strategies, we show that there are multiple ways to generate flexibility in upright corallines but not all morphological strategies are mechanically equivalent. Corallinoids have many joints, lithophylloids have pliant joints, and metagoniolithoids have longer joints—while these strategies can lead to comparable thallus flexibility, they also lead to different levels of stress amplification in bending. Moreover, genicula at greatest risk of stress amplification are typically the strongest, universally mitigating the trade-off between flexibility and stress reduction.

Key index words: bending; biomechanics; convergent evolution; coralline; flexibility; genicula; intergenicula; morphometrics

Abbreviations: CO1, cytochrome c oxidase subunit I; MPa, megapascal

Corallines are a monophyletic clade of red algae, morphologically united by their hard, calcified thalli (Johansen 1981). While algal calcification is not unique to the corallines, they are notable for comprising over 500 species that calcify without exception (Bailey and Chapman 1998, Bilan and Usov 2001, Brodie and Zuccarello 2007). Coralline species exhibit a variety of growth forms, ranging from smooth crusts to branching, upright articulated morphologies (see Johansen 1981). Between these two extremes are crusts of varying degrees of bumpiness, free-living rhodoliths, and reduced articulated corallines comprised of miniscule uprights with large crustose bases (Martone et al. 2012).

While most coralline forms are calcified throughout their entire thallus, the majority of upright species possess uncalcified joints (called genicula) that separate calcified segments (intergenicula). This feature provides flexibility to otherwise rigid thalli, allowing articulated corallines to gain flexibility comparable to that of fleshy algae. While corallines occupy oceans worldwide in habitats ranging from the intertidal zone to depths of over 200 m (Littler et al. 1986), they are particularly successful in the wave-swept intertidal and shallow subtidal habitats of rocky intertidal shores, where flexibility is considered a prerequisite for the survival of upright macroalgae (Harder et al. 2004). The importance of flexibility may have been a contributing factor to the evolution of joints in upright corallines, which has occurred at least three separate times as supported by both molecular (Bailey and Chapman 1998, Bittner et al. 2011, Kato et al. 2011) and fossil evidence (Aguirre et al. 2010, Kundal 2011). Representatives of all three articulated coralline clades—Corallinoideae, Metagoniolithoideae, and Lithophylloideae (previously Amphiroideae)—can be found in abundance in wave-swept environments. Interestingly, the structure and development of joints in the three lineages is significantly different (see Johansen 1981). While joints in articulated corallines universally aid in flexibility, we know little about how the degree of flexibility and the depth of convergence compare. While the basic jointed morphology is analogous, the structural and

developmental details are not, so what about the function?

Flexibility has consequences. For macroalgae living in wave-exposed environments, flexibility is an important drag-mitigating mechanism (Harder et al. 2004). Flexible macroalgae can bend toward the substrate, lowering the area projected into flow (Vogel 1984, Denny 1985, Martone and Denny 2008). They may also fold up blades or branches, reconfiguring passively into more streamlined shapes (Denny and Gaylord 2002, Boller and Carrington 2006, Martone et al. 2012). These shifts in size and shape reduce the amount of drag that is experienced, thereby reducing the chance of dislodgement. While the benefits of flexibility for fleshy macroalgae have been well documented (e.g., Vogel 1984, Denny and Gaylord 2002, Harder et al. 2006, Starko et al. 2015), flexibility in articulated corallines comes with unique challenges that make the benefits to flexibility and survival less apparent. By limiting bending to discrete joints, stress in joint tissue is amplified, and this stress increases as bending increases (Martone and Denny 2008).

Computational modeling of the articulated corallinoid species, *Calliarthron cheilosporioides*, revealed that morphological trade-offs exist between increasing flexibility and decreasing stress; for example, decreasing joint width might increase flexibility of the overall thallus, but it decreases the tissue available to withstand stress (Martone and Denny 2008). For this species, genicular morphology is somewhat balanced between stress mitigation and flexibility maximization (Martone and Denny 2008). An "ideal" *Calliarthron* geniculum would have a moderate increase in genicular length—this could be indicative of a developmental constraint in corallinoids, as genicular cells lose cytoplasm and organelles as they lengthen and may have some upper limit to how far they can extend (Martone and Denny 2008). Corallinoid joints are composed of only a single tier of cells, so limitation on individual cell length translates to a limitation on total genicular length (Johansen 1969, 1981, Martone and Denny 2008). Single-tiered cell structure is specific to corallinoid genicula—articulated corallines in other groups may be able lengthen their joints further by adding additional cell layers. While the model does not answer the question of why an articulated coralline possesses a certain morphology, it can be used to investigate the consequences of that morphology. Modeling is thus an ideal tool for exploring the degree to which the genicula of different articulated coralline clades are functionally convergent.

Articulated corallines are not analogous at all levels of organization. The development of genicula progresses differently in all three articulated coralline clades; corallinoid and lithophylloid genicula form via decalcification of medullary cells and a cracking of the surrounding cortex (Johansen 1969, 1981), whereas genicula in Metagoniolithoideae form when meristematic cells switch production from calcified to uncalcified cells (Ducker 1979). Additionally, while corallinoid joints are always single-tiered and anchored in intergenicular tissue at either end (Johansen 1969, 1981, Martone and Denny 2008), lithophylloid joints are typically multitiered (Johansen 1969, 1981), and metagoniolithoid joints are composed of many layers of irregularly shaped cells that lack tiered organization altogether (Ducker 1979). The cells of corallinoid genicula are unique in being very loosely connected laterally, which causes whole joints to behave more like a rope than a solid when bending (Martone and Denny 2008). Lack of attachment between neighboring cells also mitigates the propagation of cracks throughout the tissue, causing corallinoid genicula to be highly resistant to fatigue from repeated stresses (Denny et al. 2013). Lithophylloid and metagoniolithoid joint tissues, on the other hand, are much more cohesive in structure. Structural and developmental constraints may affect the degree to which each articulated clade can achieve a mechanically balanced morphology or the morphological strategy used to do so.

There are two main morphological ways in which a frond can achieve flexibility without sacrificing the capability to withstand stress—a frond can increase the number of joints or increase the length of individual joints. Both options result in a similar level of uncalcified tissue per unit length. Having thin joints can not only increase flexibility but this also comes with an increased likelihood of breakage. A fourth strategy to mitigate mechanical challenges is adjusting material properties of genicular tissue—corallinoids, lithophylloids, and metagoniolithoids all possess joints with high material strength and extensibility relative to other red algal groups (Janot and Martone 2016), which may help them resist stress amplification in their genicula. However, because there are significant differences in material properties among the three articulated groups (Janot and Martone 2016), the role of material properties in stress mitigation is uncertain.

In this study, we aimed to determine whether articulated corallines universally mitigate or resist bending stress in the same way, despite convergently evolved bending structures. Given differences in structure and development, we hypothesized that articulated lineages may employ different strategies to achieve similar levels of flexibility. Using a computational model developed by Martone and Denny (2008), we explored the morphological trade-off between resisting stress and maintaining flexibility as well as the consequences associated with different bending strategies.

MATERIALS AND METHODS

Specimen collection. Corallinoid species *Calliarthron tuberculosum*, *Corallina officinalis* var. *chilensis*, and *Johansenia macmillanii* were collected subtidally at a depth of ~3 m from

Botanical Beach (48°31′48″ N, 124°27′18″ W) on Vancouver Island, BC, Canada, in June–July 2012. Lithophylloid species *Amphiroa anceps* and *Amphiroa gracilis* were collected in Point Peron (32°16′01″ S, 115°41′14″ E), Perth, Western Australia, at depths of 3.0–4.6 m in December 2012. Both Botanical Beach and Point Peron are located on open coasts unsheltered by any surrounding land features and are generally considered "wave-exposed" locations.

Metagoniolithon stelliferum #1 and #2 are used here to denote two morphotypes that currently fall under the name *M. stelliferum*. The two morphotypes are genetically distinct from one another at a species level, based on sequencing of psbA, CO1, and rbcL genes (Janot and Martone, in prep). Both metagoniolithoid species were found growing epiphytically on *Amphibolis* seagrass in the same bed, with no obvious habitat delineation between them. They were collected from Point Peron at 3.0–4.6 m in December 2012.

Specimens were kept in cooled seawater in the laboratory for no more than 48 h prior to mechanical testing. Representative individuals of each species were deposited at the UBC Herbarium for future reference: *Calliarthron tuberculosum* (A91564), *Corallina officinalis* var. *chilensis* (A91563, as *C. officinalis*), *Johansenia macmillanii* (A91561), *Amphiroa gracilis* (A91572), *A. anceps* (A91566), *Metagoniolithon stelliferum* #1 (A91576, as *M. stelliferum*), and *M. stelliferum* #2 (A91579, as *Metagoniolithon* sp.).

Flexibility. Bending tests followed the protocol outlined by Martone and Denny (2008). Basal segments just over 2 cm in length were trimmed of all branches; for dichotomously branching species, one side of each dichotomy was removed to produce a straight, unbranched segment. Trimmed segments were mounted horizontally in a vice clamp (Fig. 1), with the first 1–3 joints held stationary within the grips. The insides of the grips were lined with neoprene and sandpaper to prevent crushing or slippage of the specimen. Dental floss was looped and tied around the segment at a point ~2 cm distal to the anchored end and fixed in place with cyanoacrylate glue. Weights of 5, 20, and 100 g (corresponding to 0.05, 0.20, and 0.98 N of force) were hung from the end of the
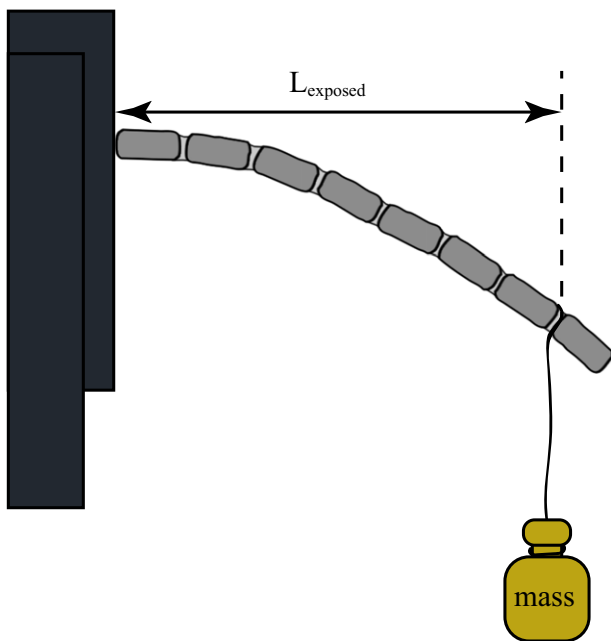
floss. Chilled seawater was pipetted onto bent segments between each load. Fifteen specimens per species were tested.

Photos were taken at each load from the side (see Fig. 1) using a digital camera, and flexibility was determined by analyzing photos in ImageJ (NIH Image, http://rsb.info.nih.gov/ij).

Flexibility was quantified by measuring the "percent bent" of the original segment length. As bending occurs, the height projected into the hypothetical flow decreases, so percent bent was measured as:

$$\text{Percent Bent} = \left(1 - \left(\frac{L_{\text{exposed}}}{L_{\text{initial}}}\right)\right) \times 100\# \qquad (1)$$

where L_{exposed} is the length projected horizontally during bending, and L_{initial} is the starting segment length (see Fig. 1).

After visualizing the data, we focused on the percent bent at 0.05 N only, as subsequent forces only caused individuals to converge upon 100%. Due to unequal variances between species, statistical comparisons were done with a nonparametric Kruskal–Wallis test and post hoc Dunn's test. Both tests were performed in R 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria) using the RStudio interface (version 0.98.1056; RStudio, Boston, MA, USA) and the dunn.test() function from the dunn.test package (Dunn's test of multiple comparisons using rank sums, version 1.2.3; Alexis Dinno 2015).

Morphometrics. The following genicular and intergenicular dimensions were measured for each bent specimen: short- and long-genicular radii, genicular length, short intergenicular radius, intergenicular length, and intergenicular lip length (corallinoids only). Corallinoid species in this study possess intergenicular lips that overlap genicula on either end (see Fig. 2A inset); this meant that corallinoid genicula had to be long sectioned to obtain an accurate measurement of genicular length, precluding those same genicula from being cross-sectioned for measurements of genicular radii. For these species, measurements of each geniculum switched between measuring genicular length or radii starting from the anchored end, and missing values were replaced with an average of the values from adjacent genicula. For species with no lips (lithophylloids and metagoniolithoids), every joint that underwent bending was measured for all parameters. In cases where genicula or intergenicula appeared to individually grow wider from the proximal to the distal end (e.g., *Amphiroa anceps*; Fig. 2E), an average of the two ends was taken. Measurements were performed under a dissection microscope (model SZ61; Olympus Canada, Richmond Hill, ON, Canada) with an attached camera (model DP20; Olympus Canada). Averages of all morphometrics were calculated for each specimen.

Metagoniolithon stelliferum #1 has a joint morphology that required extra consideration in measuring intergenicular parameters (see Fig. 2F). This species possesses unusually long meristematic genicula, with false whorls of branches that originate from the midpoint (Ducker 1979). Branching points are much thicker than the surrounding genicular tissue and did not appear to bend during tests despite being noncalcified. We thus chose to group them with intergenicula when measuring morphometrics—"intergenicular" radius and length values presented in this study for *M. stelliferum* #1 represent averages of both intergenicula and branching points and should not be considered representative of the true morphology of this species except when modeling bending behavior.

To compare similarly flexible individuals morphometrically, two ranges of flexibility were chosen: 50%–70% bending and 70%–90% bending at 0.05 N. Individuals that fell within
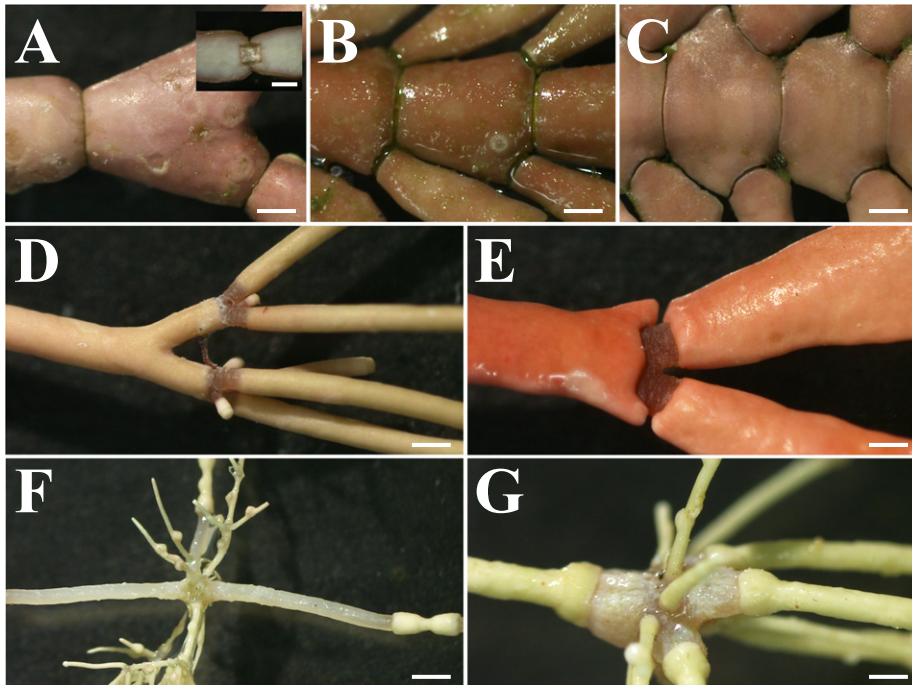
FIG. 2. Close-up of genicula and intergenicula for each species studied. (A–C) Corallinoid species, *Calliarthron tuberculosum*, *Corallina officinalis*, *Johansenia macmillanii*. (D, E) Lithophylloid species, *Amphiroa gracilis*, *Amphiroa anceps*. (F, G) Metagoniolithoid species, *Metagoniolithon stelliferum* #1 and #2. Scale bars = 0.5mm.

these ranges were averaged by species, and all morphometric parameters were compared. We also calculated and compared intergenicular length/genicular length to look at relative levels of calcification. Statistics were not applied to these qualitative comparisons, as some species had too few individuals within a given range to make statistical analysis informative.

Modeling. Bending of different species was modeled in Octave 4.2.1 (Eaton et al. 2016; https://www.gnu.org/softwa re/octave/) using code adapted from Martone and Denny (2008) to explore the consequences of using different genicular traits to achieve flexibility. Like Martone and Denny (2008), we modeled corallinoid genicula as cables, but uniquely modeled lithophylloid and metagoniolithoid genicula as solid beams. For thorough derivation of mathematical details, see appendix A of Martone and Denny (2008). For a summary of the distinction between the cable and solid models, see Appendix S1 and Figure S1 in the Supporting Information of this paper.

Bending stress. The model calculated total maximum stress in the first geniculum, with both tensile and bending stress components. The mathematical details of this are outlined in appendix B of Martone and Denny (2008). The morphometric parameters of fronds used in real bending tests were input into the model to estimate stress and to explore other mechanical consequences of morphology beyond flexibility. To separate the effects of morphology from tissue properties, all species were modeled using an "average coralline" tissue stiffness, which was calculated as the mean of species tissue stiffness values taken from Janot and Martone (2016). Corallinoid species were again modeled using a cable model, while lithophylloid and metagoniolithoid species were modeled using a solid model. Species averages of estimated stress in bending were then compared with species averages of breaking strength obtained from Janot and Martone (2016).

Mechanical consequences of morphology. The model was used to explore the individual effects of different morphometric parameters on flexibility and stress. Mean values for genicular and intergenicular length and width of each species were calculated from the measurements taken during bending tests.

These values were used to virtually build an "average" 2 cm tall frond of each species—genicula in a single frond were assumed to have constant morphology from base to tip. Virtual frond data were input into the model and tested at a force of 0.05 N, and both percent bent (Eq. 1) and stress in the first geniculum (MPa) were quantified. Each morphometric parameter was then varied while holding all other values constant, with flexibility and stress being recorded for each trial.

Genicular radii were varied together to explore the overall effect of joint width. The amount to which each parameter could be varied differed depending on other morphological constraints, which in turn depended on the species being modeled. Genicula were never modeled as being wider than surrounding intergenicula, except in the case of *Metagoniolithon stelliferum* #2, a species in which this naturally occurs (Fig. 2G). All fronds were held at a 2 cm length as joint morphology varied, so variation in intergenicular length was limited to different extents in different species, due to the need to maintain at least one geniculum within the modeled frond. As with our morphometric comparisons, non-bending branching points of *M. stelliferum* #2 were grouped with intergenicula. Percent change in each genicular/intergenicular dimension was graphed against both total stress in the first geniculum (MPa) and percent bent—genicular dimensions were considered "balanced" when low stress and high flexibility coincided.

RESULTS

Flexibility. Average flexibility (as measured by percent bending at 0.05 N) depended significantly on clade (Kruskal–Wallis test followed by post hoc Dunn's test, $H_6 = 75.55$, $P < 0.001$; Fig. 3A). Metagoniolithoids generally bent more than lithophylloids, which bent more than corallinoids for a given force. *Metagoniolithon stelliferum* #1 was more flexible than all other species, with an average percent

Fig. 3. (A) Flexibility ranges of articulated coralline species measured as the percent bent with the application of 0.05 N of force. Each point represents one individual. Corallinoid species are represented by green squares, lithophylloid species by purple triangles, and metagoniolithoid species by orange circles. Bold lowercase letters indicate the results of a nonparametric Kruskal–Wallis test and post hoc Dunn's test ($P < 0.001$). (B, C) Average morphometrics of corallines that bent between 50%–70% (B) and 70%–90% (C) of their initial length with the application of 0.05 N of force. Numbers above each column in subpanels B, I. and C, I. indicate the number of individuals measured for each species within a given flexibility range. Error bars represent SE.
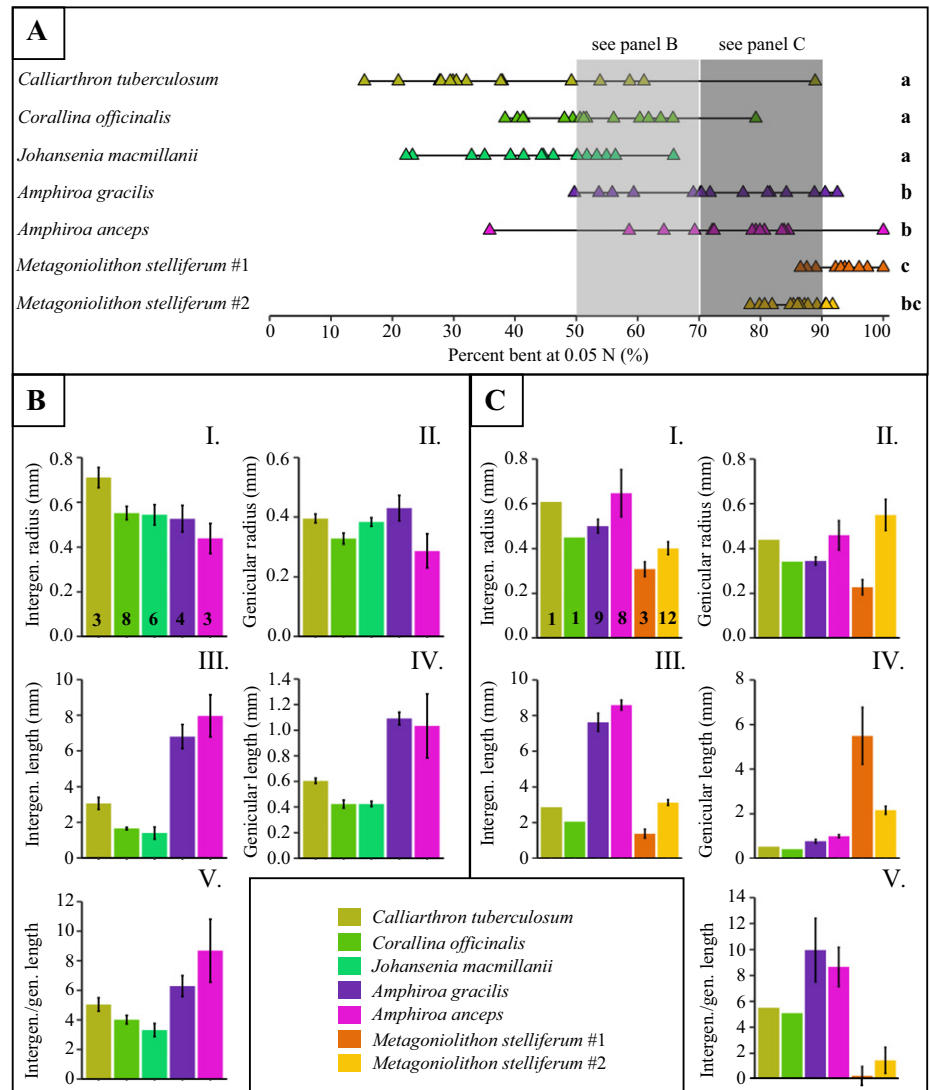
bending (mean ± SE) of 94.4% ± 1.2%. *Metagoniolithon stelliferum* #2 overlapped with *M. stelliferum* #1 and both lithophylloid species, bending 85.8% ± 1.1%. *Amphiroa anceps* and *A. gracilis* bent 76.2% ± 4.1% and 73.0% ± 3.6%, respectively. *Johansenia macmillanii* bent 44.1% ± 3.1%, *Corallina officinalis* bent 53.3% ± 2.9%, and *Calliarthron tuberculosum* bent 40.1% ± 4.9%.

Morphometrics. Specimens that bent 50%–70% exhibited different morphological characteristics, depending on subfamily. All three corallinoid species and both lithophylloid species had individuals that bent 50%–70% with an application of 0.05 N. No clear pattern was observed in either intergenicular or genicular radii (Fig. 3B, I–II). Both intergenicular and genicular lengths were higher in lithophylloid species than in corallinoid species (Fig. 3B, III–IV). Intergenicular length/genicular length was also higher in lithophylloids than corallinoids (Fig. 3B, V), indicating more calcified tissue per unit length.

Almost all species in this study had at least one individual that bent within the 70%–90%, excepting *Johansenia macmillanii*. Again, strategies for achieving this level of bending appeared to differ depending on subfamily. No clear pattern was observed in intergenicular or genicular radii (Fig. 3C). Lithophylloids once again had the longest intergenicula (Fig. 3C, III), whereas both metagoniolithoid species had notably long genicula (Fig. 3C, IV). Both metagoniolithoids had a very low level of calcification per unit length (Fig. 3C, V).

Bending stress. Modeled total stress in the first geniculum at 0.05 N was highest in the corallinoids (Fig. 4): we predicted *Calliarthron tuberculosum* would experience 20.3 ± 4.5 MPa of stress, *C. officinalis* would experience 16.6 ± 3.8 MPa, and *J. macmillanii* would experience 11.0 ± 3.4 MPa. Modeled total stress was higher in lithophylloids than metagoniolithoids, with *Amphiroa gracilis* predicted to experience 7.03 ± 0.5 MPa and *A. anceps* predicted to experience 10.7 ± 2.4 MPa. The model suggests
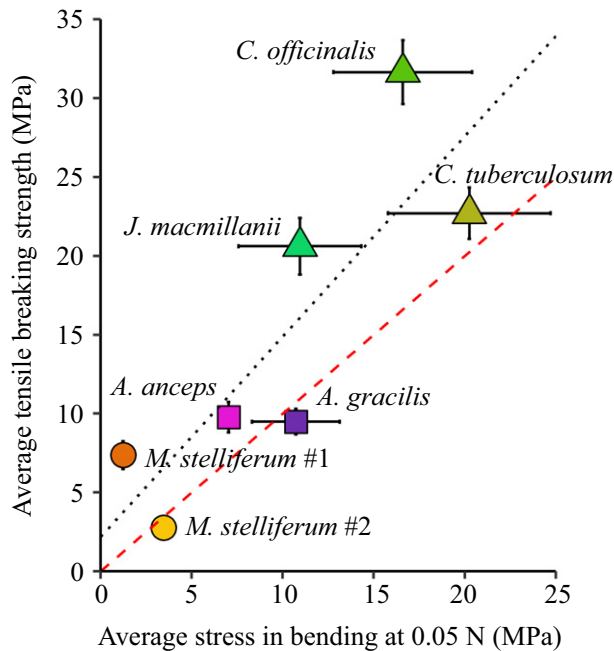
FIG. 4. The relationship between the material stress experienced in bending and the material strength of genicular tissue in articulated coralline species. Points represent species averages ($n = 15$), and error bars represent SE. Corallinoid species are shown in shades of green, lithophylloid species are shown in shades of purple, and metagoniolithoids are shown in shades of orange. The black dotted line represents a line of best fit ($y = 1.3x + 2.2$, $R^2 = 0.71$), and the red dashed line represents a 1:1 line.

that metagoniolithoids incur the least amount of stress, with *Metagoniolithon stelliferum* #1 predicted to experience 1.2 ± 0.1 MPa and *M. stelliferum* #2 predicted to experience 3.5 ± 0.4 MPa. Species average stress in bending was positively correlated with species average tissue strength ($y = 1.3x + 2.2$, $R^2 = 0.71$; Fig. 4). In almost all cases, tissue strength was greater than modeled stress: *M. stelliferum* #2 was the one exception, with a tissue strength of 2.8 ± 0.3 MPa, 0.7 MPa lower than the stress it was expected to incur (Fig. 4).

Mechanical consequences of morphology. Intergenicular radius appeared to be close to balanced for most species, in that a decrease or increase would either increase stress or decrease flexibility with minimal benefit in the other parameter (Fig. 5). *Calliarthronv tuberculosum* (Fig. 5A) was an exception and could drastically decrease stress with a minimal decrease in flexibility by decreasing intergenicular radius even slightly. According to the model, *Amphiroa anceps* (Fig. 5E) could increase flexibility slightly by increasing intergenicular radius, but this would also come with an increase in stress.

The consequences of varying genicular radius were variable between species (Fig. 6). *Corallina officinalis* (Fig. 6B), *Amphiroa anceps* (Fig. 6E), and *Metagoniolithon stelliferum* #2 would increase in flexibility with almost no effect on stress by possessing a

slightly smaller genicular radius. *Calliarthron tuberculosum* (Fig. 6A) and *Johansenia macmillanii* (Fig. 6C) could decrease stress by increasing genicular radii, although the associated decrease in flexibility would be equally significant. *Amphiroa gracilis* (Fig. 6D) and *M. stelliferum* #1 (Fig. 6F) were both largely unaffected by minor shifts in genicular radii, although a large decrease would result in a slight increase in stress.

Calliarthron tuberculosum would lower stress while maintaining high flexibility by possessing a slightly lower intergenicular length (Fig. 7A). All other species appeared to have balanced intergenicular lengths (Fig. 7, B–G): in the case of the lithophylloid and metagoniolithoid species, shifting length positively or negatively within the range tested would have minimal mechanical effect. *Calliarthron tuberculosum* (Fig. 8A), *Johansenia macmillanii* (Fig. 8C), *Amphiroa anceps* (Fig. 8E), and *Metagoniolithon stelliferum* #2 would all increase flexibility and decrease stress by increasing genicular length. *Corallina officinalis* (Fig. 8B), *A. gracilis* (Fig. 8D), and *M. stelliferum* #1 (Fig. 8F) had sufficiently long genicula to both maximize flexibility and minimize stress, with little to be gained from a further increase in genicular length.

DISCUSSION

Articulated corallines may have convergently evolved to possess genicula, but not without significant variation on the overall theme. Although species differed in average levels of bending, there was sufficient overlap to allow for morphological comparisons of similarly flexible individuals. From these comparisons, a pattern emerges, wherein each subfamily utilizes a different strategy to achieve flexibility. Both corallinoids and metagoniolithoids have joint morphologies that minimize the amount of calcification per unit length, but in different ways. Corallinoids have many genicula, as evidenced by the fact that a low calcification level is maintained despite genicula being relatively short. Metagoniolithoids, in contrast, have a smaller number of longer genicula. The lithophylloid strategy is not immediately apparent based on morphology alone—while lithophylloid genicula were longer than corallinoid genicula, so too were lithophylloid intergenicula, resulting in a high proportion of calcified tissue per unit length compared to both corallinoids and metagoniolithoids. It is here that material properties become relevant; average genicular tissue stiffness is 11 MPa in *Amphiroa gracilis* and 12 MPa in *A. anceps*, less than half the stiffness of any corallinoid species tested (29–52 MPa; Janot and Martone 2016). This allows lithophylloids to maintain a higher level of calcification than other groups without sacrificing the capability to bend in flow.

Differences in bending strategy may reflect different developmental and structural constraints. As
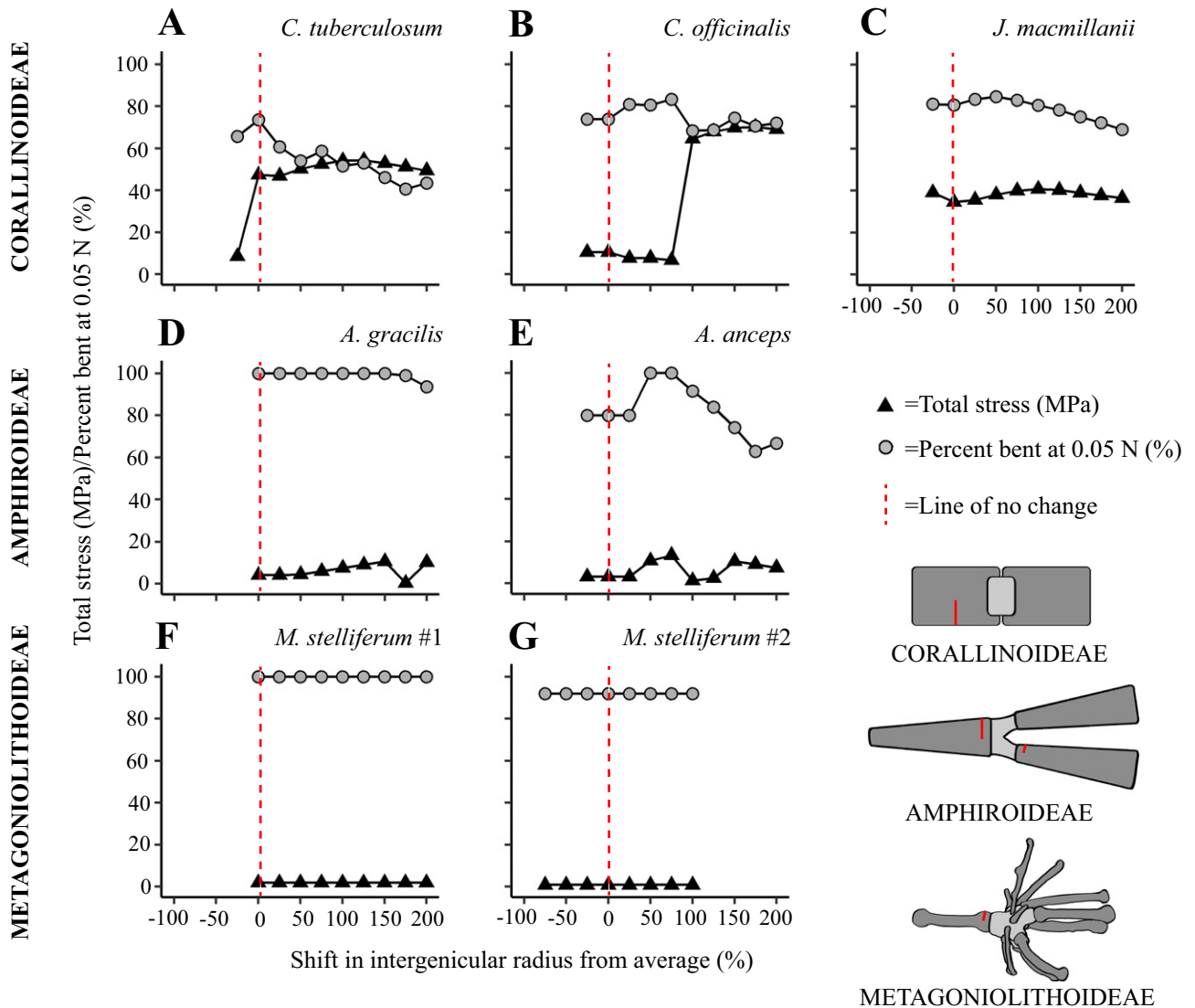
FIG. 5. The effect of varying intergenicular radius on the maximum stress experienced in the first geniculum (black triangles) and percent bent (gray circles) with the application of 0.05 N. Lines of no change (dotted red lines) indicate stress and flexibility for an "average" individual of a given species. Typical joint construction of each articulated clade is illustrated in the bottom right—solid red bars indicate where intergenicular radius would be measured.

previously noted, genicula in corallinoids likely have an upper limit to how long they can grow, due to being composed of a single tier of elongated cells with minimal cellular content. This means that increasing the number of genicula may be the only option corallinoids have available to increase the overall proportion of genicular tissue. The multizonal structure of lithophylloid and metagoniolithoid genicula makes limitations on individual cell length irrelevant to genicular length. In addition, metagoniolithoid genicula are meristematic and often continue to grow throughout the life of the frond (Ducker 1979), so it is perhaps not surprising that the metagoniolithoid species tested here achieved flexibility primarily by having long genicula. The reasoning behind the low stiffness of

lithophylloid genicula is unclear; it may be that morphological constraints necessitate low stiffness to allow for bending, or it may be that low stiffness allows for a more calcified morphology. Morphological and mechanical tests on a greater number of lithophylloid species might help clarify this relationship.

Comparisons of expected bending stress also revealed a relationship between morphology and material properties. Species that are expected to incur the most stress in bending due to morphology are also composed of stronger tissues that can withstand that stress. This is at least partially due to secondary cell wall growth in the corallinoids, which fortifies the primary cell wall and increases the quantity of material resisting stress (Martone 2007,
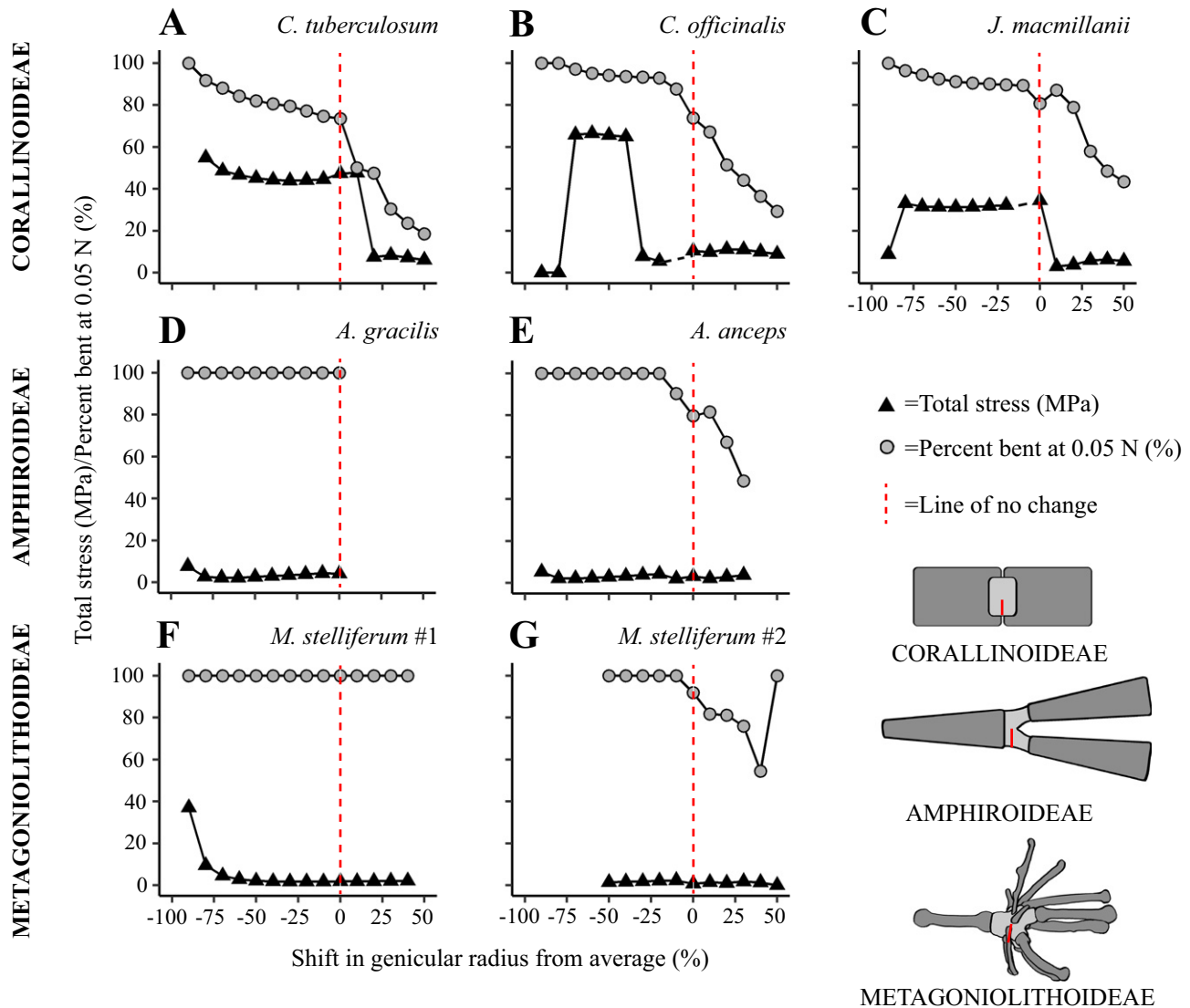
FIG. 6. The effect of varying genicular radius on the maximum stress experienced in the first geniculum (black triangles) and percent bent (gray circles) with the application of 0.05 N. Lines of no change (dotted red lines) indicate stress and flexibility for an "average" individual of a given species. Dotted line breaks in B and C indicate regions where a data point was skipped, due to a mathematical anomaly in the MatLab model. Typical joint construction of each articulated clade is illustrated in the bottom right—solid red bars indicate where genicular radius would be measured.

Janot and Martone 2016). The chemical composition of genicular cell walls in the three articulated clades is also likely to play a role, but only *Calliarthron cheilosporioides* joints have been explored for this thus far (see Martone et al. 2009, 2010). As with the properties of lithophylloid genicula, a "chicken or egg" question arises; are corallinoid genicula strong because they cannot grow longer to minimize bending stress or does joint morphology represent a "bare minimum" of joint formation that is possible because of genicular strength?

Shifting one genicular parameter at a time using modeling revealed that corallinoids are not necessarily well balanced between flexibility and stress. *Calliarthron tuberculosum* specifically could benefit

from minor changes in morphology—indeed, the average modeled frond for this species appears to be right on the edge of drastically reduced stress for every morphometric investigated. *Johansenia macmillanii* is also on the detrimental side of a tipping point for genicular radii, intergenicular length, and genicular length. In contrast, the average *C. officinalis* consistently minimizes bending stress, which is notable given that it is also composed of the strongest material. It is possible that *C. officinalis* is "overdesigned" and could survive with weaker tissue—however, no part of this analysis considered frond size, density, or habitat, which could impact the amount of drag experienced in the field. Although all species in this study were collected from wave-
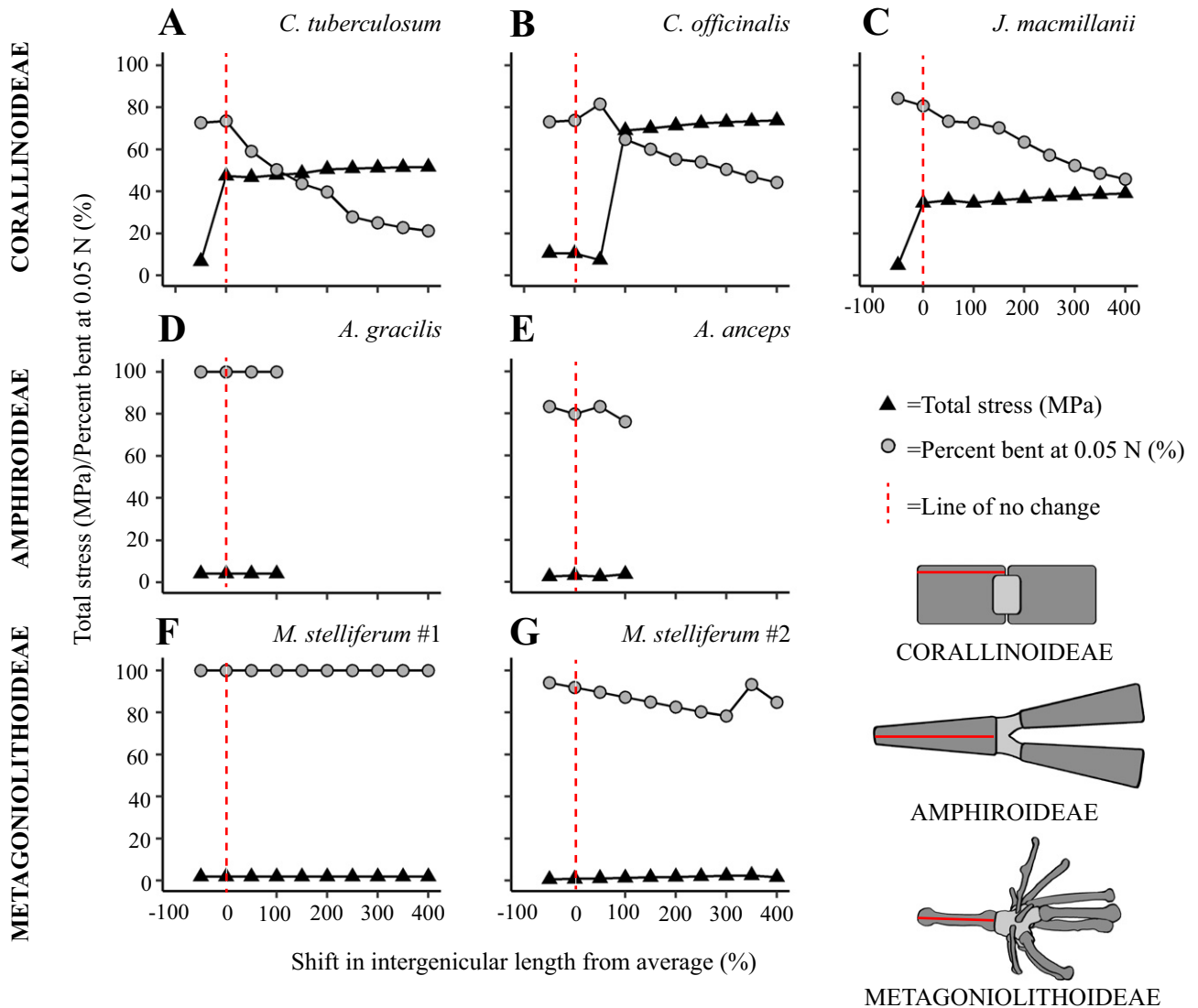
Fig. 7. The effect of varying intergenicular length on the maximum stress experienced in the first geniculum (black triangles) and percent bent (gray circles) with the application of 0.05 N. Lines of no change (dotted red lines) indicate stress and flexibility for an "average" individual of a given species. Typical joint construction of each articulated clade is illustrated in the bottom right—solid red bars indicate where intergenicular length would be measured.

swept environments, if *C. officinalis* lives in a particularly wave-exposed microhabitat or assumes a more drag-prone morphology, then it may experience more drag than other species nearby and may need stronger tissues to survive.

Modeled lithophylloid and metagoniolithoid species consistently maintained high flexibility and low stress, although in some cases, small shifts in genicular morphology could improve flexibility even farther without a detrimental effect on stress. Perhaps, maximal flexibility is not necessarily the most ideal situation for all upright corallines, as excess flexibility might cause coralline thalli to flop over when it is not mechanically necessary, that is, when wave action is minimal. This would directly contradict many of the benefits associated with upright growth,

such as increased area available for photosynthesis and nutrient uptake, or avoidance of herbivores (Lubchenco and Cubit 1980, Padilla 1984). Given that each articulated clade exhibited a different flexibility range in bending tests, it is unlikely that the optimal level of flexibility is the same for all species or environments.

Two of the three corallinoid species could theoretically reduce stress by reducing the proportion of calcified tissue present, either by shortening intergenicula (Fig. 7) or lengthening genicula (Fig. 8)—this suggests some nonmechanical benefit to heightened calcification levels. That is, if decreasing calcified tissue could generally increase flexibility and decrease stress, then why remain calcified? While calcification has typically been considered a
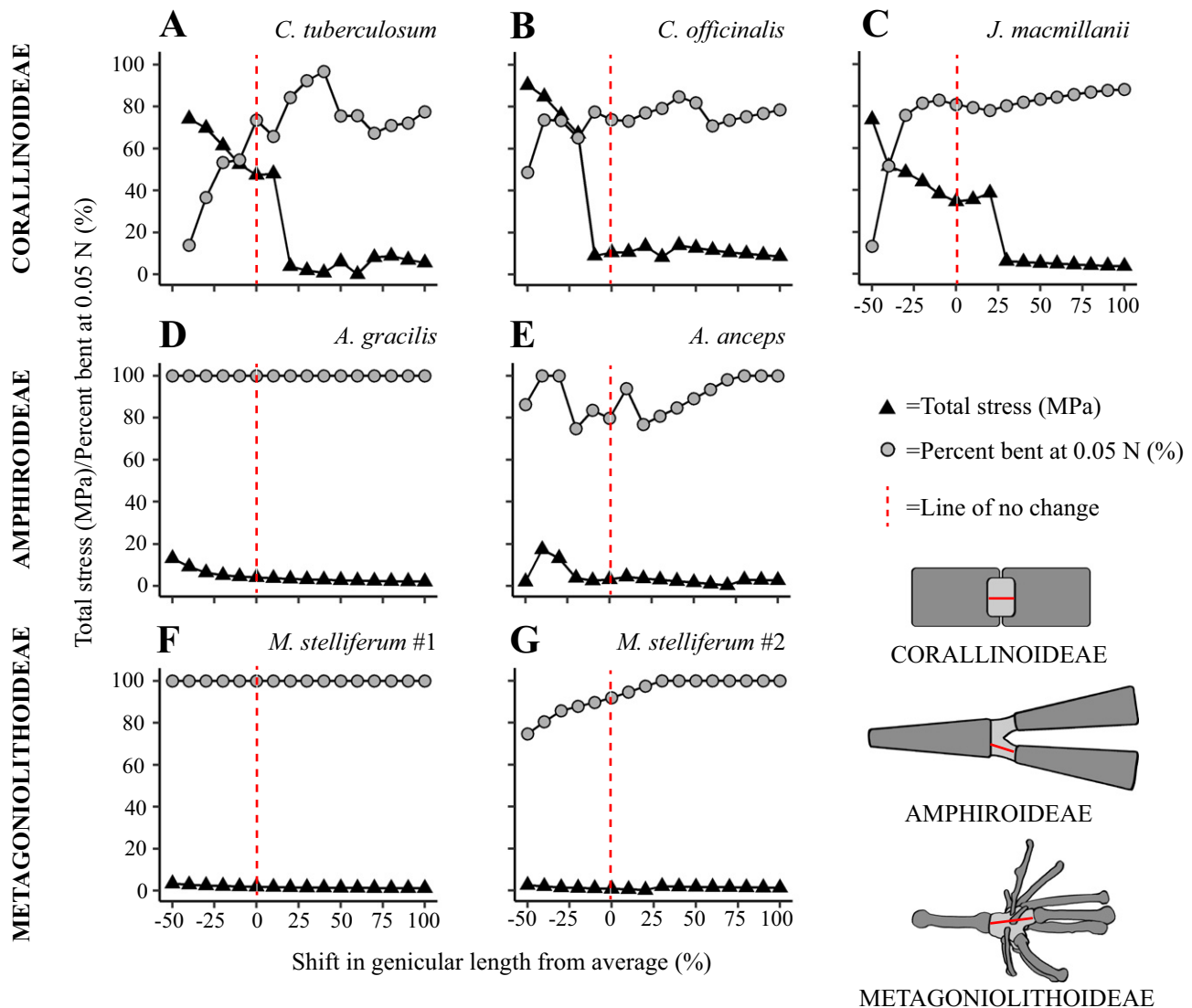
FIG. 8. The effect of varying genicular length on the maximum stress experienced in the first geniculum (black triangles) and percent bent (gray circles) with the application of 0.05 N. Lines of no change (dotted red lines) indicate stress and flexibility for an "average" individual of a given species. Typical joint construction of each articulated clade is illustrated in the bottom right—solid red bars indicate where genicular length would be measured.

deterrent against herbivory (Littler and Littler 1980, Steneck and Watling 1982, Pennings and Paul 1992), this is largely dependent on the type of herbivore and it is associated feeding apparatus (Padilla 1989, Maneveldt and Keats 2008). Indeed, some degree of herbivory may help corallines thrive, as it facilitates the removal of epiphytes and fouling organisms from the epithallial surface (Steneck 1982, Littler et al. 1995, Berthelsen and Taylor 2014). While calcification may not completely prevent herbivory, it could provide just enough resistance to limit the depth of grazing that occurs or to facilitate rapid recovery (Steneck et al. 1991). Calcification also provides protection for reproductive propagules, which are housed in cavities called "conceptacles" that reside exclusively on the calcified intergenicula (Johansen 1981), so increased

intergenicular tissue could also correspond with an increased surface area available for reproductive activity. Most of the studies done on the benefits of calcification in corallines have been performed on crustose forms, and its adaptive significance in articulated corallines deserves further study.

CONCLUSION

Genicula in articulated corallines may have evolved convergently from similar environmental pressures, but it has led to a variety of morphological forms. Different species utilize different strategies in response to the mechanical challenges inherent in being articulated, even when comparing within a set range of flexibility. Determining whether these strategies are consistent within each

articulated clade requires further testing on additional species, particularly within the lithophylloids and metagoniolithoids. Morphological types are not equal in balancing flexibility with bending stress, raising additional questions about the developmental constraints imposed on genicula, the need for flexibility, and the benefits of calcification. Biomechanical challenges caused by morphology may be partially mitigated with material properties—the stressful morphology of corallinoids is offset by strong tissues, while the inflexible morphology of lithophylloids is offset by pliant tissues. Structural and computational biomechanics can help clarify at which level of organization convergent evolution has occurred in ostensibly similar structures.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Genicula bending in long section before intergenicular contact (A, B), at the moment of contact (C, D), and after contact (E, F). Both cable (A, C, E) and solid (B, D, F) models are shown. Light gray represents uncalcified genicular tissue; dark gray represents the ends of adjacent calcified intergenicula. See Martone and Denny (2008) for additional derivations and details.

Appendix S1. Details of the solid and cable bending models.